3

Escape Reflexes in Earthworms and Other Annelids

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1. Introduction

Escape or startle reflexes are characteristically seen in many representatives of the Phylum Annelida (segmented worms), a group that comprises the earthworms, aquatic oligochaetes, leeches, and marine bristle or polychaete worms. From the standpoint of defense, the escape reflex represents one of the most important components of a worm's repertoire of locomotory behavior that, depending on the species, may also include undulating swimming or peristaltic creeping movements. The underlying control and coordination for all of these locomotory movements are carried out by the worm's central nervous system, which consists of a dorsal brain in anterior segments and a ventral nerve cord, the latter being composed of a chain of segmentally arranged ganglia joined by longitudinal connectives.

Although there is considerable diversity in the habitats and lifestyles of those annelid worms that possess escape reflexes, there appear to be a number of properties commonly shared in their escape reflex organization and function. These include (1) triggering of the reflex by sudden onset of threatening stimuli, (2) activation of giant nerve fibers within the ventral nerve cord, and (3) initiation of a short latency motor response, especially a synchronous, multisegmental shortening that effectively withdraws the animal from the source of the stimulus. Although one purpose

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of this chapter is to present a general overview of the organization of annelid escape reflexes, the main intent and therefore greatest emphasis will be placed on the design and function of rapid reflexes in oligochaete earthworms. Previous reviews that generally pertain to the earthworm or annelid escape reflexes include those by Laverack (1963), Bullock and Horridge (1965), Mill (1975, 1982), Gardner (1976), and Dorsett (1978, 1980).

1.1. Evidence That Giant Nerve Fibers Mediate Rapid Escape

Nicol (1948*c*) defined giant nerve fibers as "those fibres which have a much greater size than any other nerve-fibres of the animal" and "form an essential nervous component of a system which effects a rapid, widespread and synchronous response." But what is the evidence that giant nerve fibers are indeed essential and responsible for the initiation of rapid escape, in other words, that they function as so-called "command" neurons whose activity is both necessary and sufficient for the initiation of escape behavior (Kupfermann and Weiss, 1978)?

To demonstrate the criterion of necessity, the behavior should be abolished by removal of the giant fibers from the system. This has effectively been demonstrated by selectively severing giant fibers while sparing the rest of the ventral nerve cord in polychaetes (Nicol, 1948*a*), as well as in earthworms (Yolton, 1923; Stough, 1930; Ten Cate, 1938; Bullock, 1945*a*; Rushton, 1946). In all cases rapid escape movements initiated by tactile stimulation at one end of the animal progressed up to but not beyond the site of giant fiber lesion. Reversibility of this effect has also been demonstrated. That is, the recovery time required before rapid escape movements can again progress beyond the lesion corresponds to the time required for giant fiber regeneration, as determined by anatomical and physiological criteria (Bovard, 1918; Birse and Bittner, 1976, 1981; Pallas and Drewes, 1981).

Another study that pertains to the criterion of necessity is Bullock's (1948) systematic survey of 15 polychaete species in which correlations were made of giant nerve fiber morphology, electrophysiology, and associated escape behavior. In all ten species possessing giant fiber systems, as evidenced by histological and physiological observations, startle responses were significantly more pronounced than in the five species lacking such systems. Finally, it has been noted that establishment of giant nerve fiber pathways during embryonic development, as indicated by histological (Prosser, 1933) and electrophysiological observations (O'Gara *et al.*, 1982), correlates with the onset of rapid escape capabilities during the same stage of development.

Kupfermann and Weiss' (1978) criterion for sufficiency is satisfied if artificial stimulation of giant fiber spiking evokes a behavioral response indistinguishable from the response to an identical pattern of giant fiber spiking evoked by natural stimulation. This stringent criterion has not been dealt with in a rigorous fashion in any previous studies of rapid escape responses in annelids, although a number of studies indicate that some components of rapid escape, such as rapid tail flattening in the earthworm (Pallas and Drewes, 1981) and rapid longitudinal withdrawal in polychaetes and earthworms (Bullock, 1948; Horridge, 1959; Roberts, 1962*a,b,* 1966) can be evoked by just-threshold electrical stimulation of the giant nerve fibers. However, it has not yet been demonstrated whether such responses to artificial stimulation of giant fibers are indistinguishable from responses to natural stimulation of the fibers.

1.2. Experimental Utility of Annelid Escape Reflexes

By virtue of the metameric body plan of annelids, some species having hundreds of body segments, the neuronal networks underlying escape are typically arranged as sets of large, identifiable and serially homologous neurons. The large size and identifiability of neurons provide obvious advantages for studying neuronal connections in escape reflex circuitry using intracellular microelectrodes. The serially homologous arrangement of neurons presents opportunities for studying physiological and behavioral specializations related to this arrangement, such as (1) intersegmental integration of parallel sensory inputs, (2) conduction properties within intersegmental neuronal networks, and (3) segmental or regional variations in the patterns and coordination of motor responses.

An additional opportunity stems from the capability of noninvasively recording spiking activity from at least some of the neuronal units mediating rapid escape in annelids. In earthworms the capability of recording medial giant fiber spiking activity through the body wall of intact worms was first noted by Rushton and Barlow (1943). With further development of this approach, it has been demonstrated that spikes, not only from the medial giant fiber (MGF) but also from the lateral giant fibers (LGF) and associated giant motor neurons, can be resolved in surface recordings from intact unrestrained worms (Drewes *et al.*, 1978, 1980; Drewes and McFall, 1980). As shown in Figure 1, stereotyped patterns of all-or-none MGF and LGF spiking activity can be readily detected from the ventral surface of intact segments in response to tactile stimulation at either end of the animal. These stereotyped patterns of MGF and LGF activity are consistently seen not only in recordings from all specimens of the same species, but also in different species from a number of earthworm families

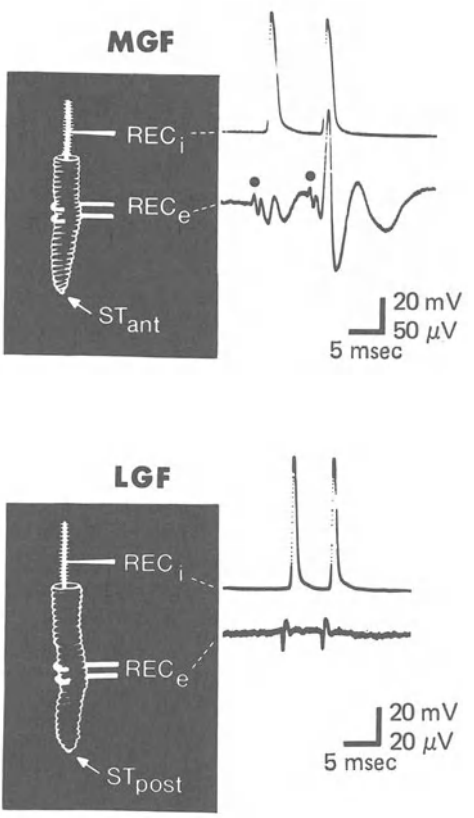


Figure 1. Simultaneous recordings of intracellular and surface electrical activity from the giant nerve fibers in two earthworm species. (Above) A pair of medial giant fiber (MGF) spikes evoked by light tactile stimulation (ST_{ant}) of head segments in *Eisenia foetida*. The two MGF spikes (•) were detected first by the pair of large hook electrodes (REC_e) in contact with the ventral surface of intact segments. The deflections following each extracellularly recorded MGF spike represent giant motor neuron and longitudinal muscle potentials (see Section 4.4). The same two MGF spikes were detected further posteriorly in intracellular microelectrode recordings (REC_i) from the exposed ventral nerve cord. (Below) A pair of lateral giant fiber (LGF) spikes evoked by tactile stimulation (ST_{post}) of posterior segments in *Lumbricus terrestris*. The two all-or-none LFG spikes were first detected extracellularly (REC_e) from the ventral surface of intact posterior segments, and then intracellularly (REC_i) from the LGF in the exposed ventral nerve cord in more anterior segments. For the intracellular recordings, the upward deflection is positive. For the MGF extracellular recording (lower trace), the upward deflection is negative whereas for the LGF extracellular recording (lower trace), the downward deflection is negative (courtesy of E. P. Vining, unpublished data).

(Figures 2 and 3). From an experimental standpoint, capabilities for noninvasive recording of giant fiber activity are useful because one can obtain a data base of direct correlations between nervous system activity *in vivo* and behavioral performance of the intact animal. Furthermore, repeated testing of the same animals is possible, thus permitting long-term studies of neural correlates of behavioral plasticity or escape reflex development.

2. Polychaete Escape Reflexes

A remarkable diversity of giant fiber number, size, and general design is found in the polychaetes (for reviews, see Nicol, 1948*b*; Bullock and Horridge, 1965). Nevertheless, all polychaete giant fiber systems seem to

be classifiable into a few basic morphological patterns. According to Dorsett (1978, 1980), these include:

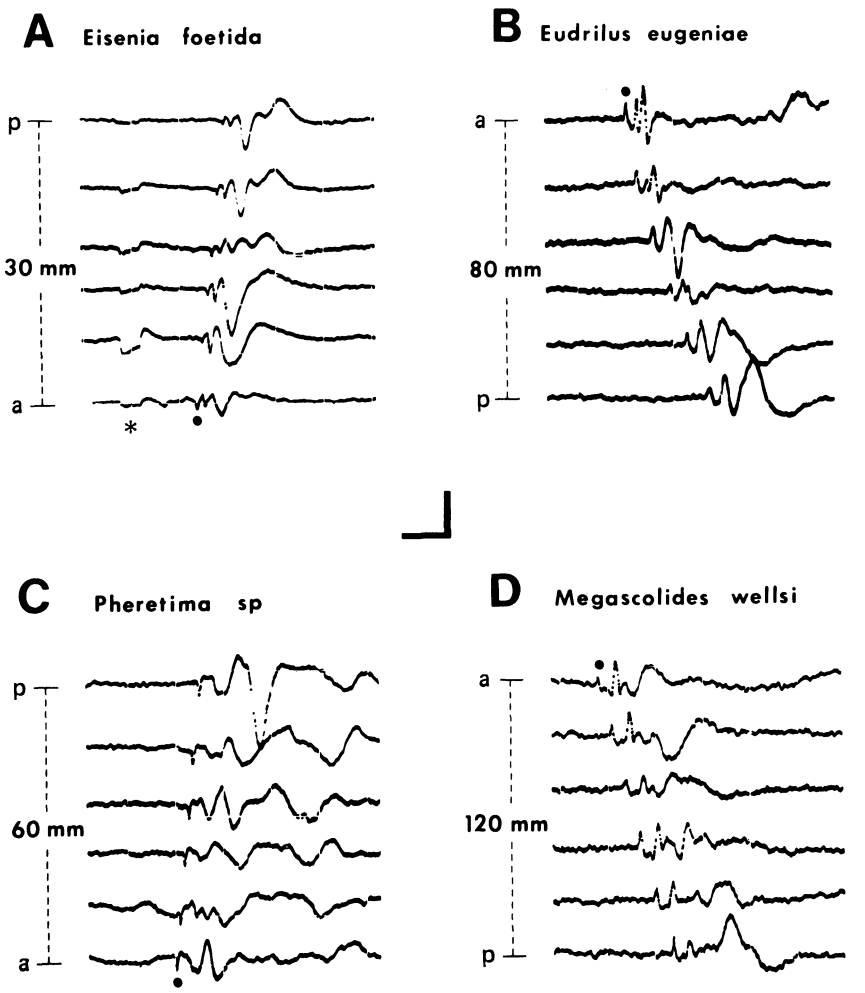
1. Unicellular fibers in which a neuron cell body gives rise to a large caliber axon that runs the length of the ventral nerve cord (e.g., giant fibers of *Protula*)
2. Multicellular fibers derived from serially homologous neurons whose enlarged axons either fuse longitudinally or form septate junctions with corresponding axons in adjacent segments (e.g., lateral giant fibers in *Nereis*)
3. Multicellular fibers derived from the lateral fusion of axons whose cell bodies originate in the same ganglion (e.g., medial giant fiber of *Nereis*)

In each case the design of the fiber appears well-suited for function as a single unit capable of rapid, uninterrupted conduction along the ventral nerve cord.

2.1. Afferent Pathways

Depending on the species, several stimulus qualities have been shown effective in evoking giant nerve fiber activity and associated escape behavior. These include tactile stimulation (e.g., light touch or body wall deformation), substrate vibration, noxious chemicals, and abrupt onset of light or shadow (Nicol, 1948*a*, 1950; Dorsett, 1964; Gwilliam, 1969; Seymour, 1972). However, measurements of the time from stimulus onset until initiation of giant fiber spiking have not been made in most species, a notable exception being *Branchiomma vesiculosum* in which the latency from touching until giant fiber spiking was 7–8 msec (Krasne, 1965). This time represents less than one half of the total reflex time in the animal.

Tactile sensory fields have been determined in a number of polychaete species (Bullock, 1945*b*, 1948, 1953; Horridge, 1959; Dorsett, 1964). Depending on the species and the specific giant fiber system, these sensory fields may be restricted to specific body regions or may involve virtually all segments of the animal. For example, in *Nereis,* the medial and paramedial giant fiber systems are selectively activated by touching anterior and posterior portions of the body, respectively, whereas the lateral giants are activated by touch anywhere along the body (Horridge, 1959). Thus it is apparent that significant overlapping can occur with respect to the sensory fields of different giant fiber systems in the same animal. However, it is not yet clear whether any given sensory unit in a segment can form divergent inputs onto two functionally distinct giant fiber systems. Also, it is not known whether inputs from tactile sensory units synapse


A Eisenia foetida
p
30 mm
a
B Eudrilus eugeniae
a
80 mm
p
C Pheretima sp
p
60 mm
a
D Megascolides wellsi
a
120 mm
p

directly onto giant fibers or whether inputs are preprocessed by nongiant interneurons interposed between sensory and giant fibers.

Whatever the specific arrangements for afferent to giant fiber transmission may be, it is clear that this transmission process is highly labile, thus providing a likely basis for the rapid habituation of escape responses to repeated stimuli. For example, in sabellid and nereid polychaetes, repetition of abrupt decreases in light intensity (Nicol, 1950; Gwilliam, 1969) or repeated tactile stimulation (Horridge, 1959; Krasne, 1965) caused rapid failure to evoke giant fiber spikes and escape withdrawal. Habituation of escape behavior and its possible adaptive significance have been discussed by Nicol (1950) and Dyal (1973). They suggested that the consequence of a worm's failure to eventually habituate to adequate but otherwise unimportant stimuli may be starvation or suffocation, especially in the case of certain tube-dwelling worms.

2.2. Central Conduction

An important factor contributing to the short latency with which escape responses can occur is rapid impulse conduction along the giant fibers. The highest values of conduction velocity for any polychaete giant fiber appear to be 20 m/sec in *Myxicola* (Nicol, 1948*b*). This fiber is 1000 μm in diameter, a relatively enormous size that has permitted extensive investigation into giant fiber membrane properties and excitability (for

Figure 2. Patterns of MGF spiking activity from four different species of intact, adult earthworms; family names are (A) Lumbricidae, (B) Eudrilidae, and (C, D) Megascolecidae. In all cases the recordings were obtained noninvasively from six recording sites along each animal by means of a printed circuit board consisting of pairs of differential recording electrodes in contact with the ventral surface of the animal (for methods, see O'Gara *et al.*, 1982). (A) A brief mechanical stimulus was applied to the anterior end of the worm using a fine glass rod attached to a small speaker. The speaker was driven by a 2-msec pulse from an electronic stimulator (*, stimulus artifact). The stimulus evoked a single, all-or-none MGF spike (•). In all records (A–D) the potentials that consistently followed a few milliseconds after each MGF spike probably represent giant motor neuron and longitudinal muscle potentials (see Section 4.4). (A) The MGF spike was progressively detected at each of the six recording sites along the animal as the spike conducted from anterior (a, bottom trace) to posterior (p, top trace). (B–D) A single MGF spike (•) was initiated in response to a very light tactile stimulus applied with a hand-held probe to the anterior end of the worm. The conduction velocites of the MGF spikes (A–D) were 11, 9, 25, and 15 m/sec, respectively. In this and later figures of this type, spike polarity depends on the direction of axonal conduction relative to the electrode grid: (A, C) The direction of propagation is from the bottom to the top trace; (B, D) from top to bottom. Thus these extracellular spikes, which are negative going, appear as (A, C) downward deflections, and (B, C) upward deflections. Voltage scale: (A) 50 μV (B, C) 35 μV, and (D) 20 μV. Time scale: 5 msec.

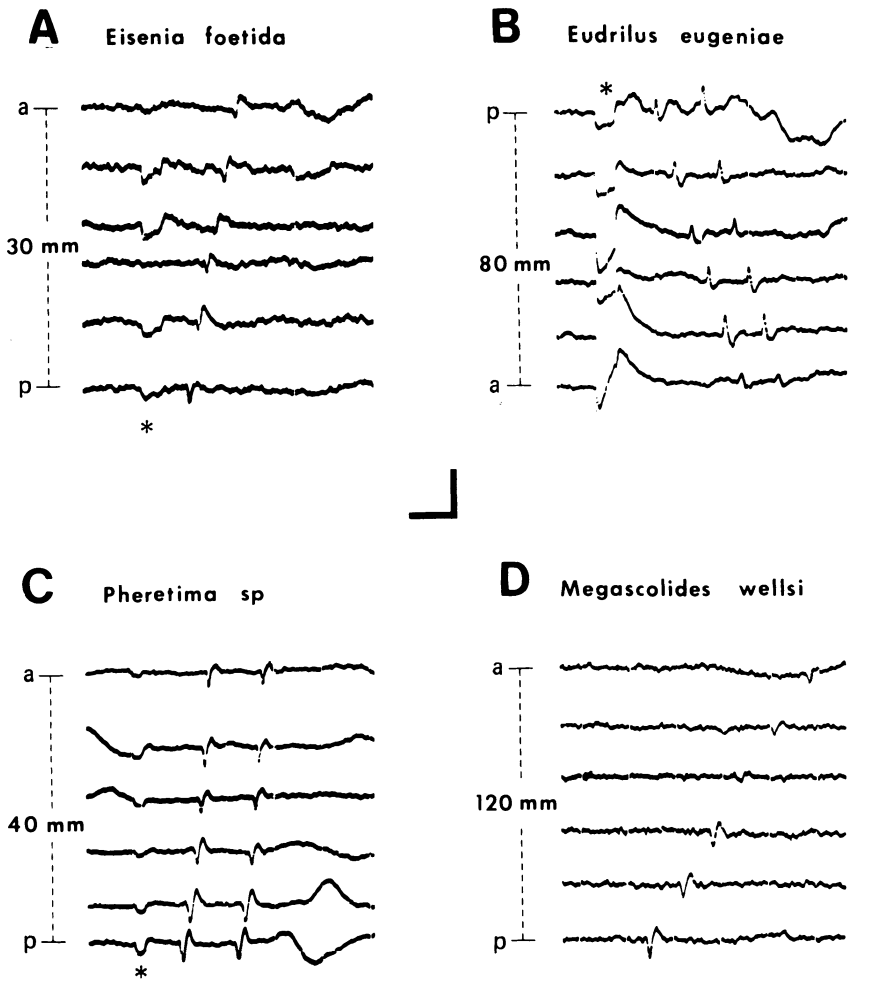


Figure 3. Patterns of LGF activity from four different species of intact, adult earthworms. (A–C) All-or-none LGF spikes were initiated by a brief mechanical stimulus applied to the posterior end of the animal with a fine glass rod attached to a speaker (*, stimulus artifact). For other details of stimulating and recording methods, see Figure 2. (D) The LGF spike was evoked by applying a light tactile stimulus to the posterior end with a hand-held probe. Conduction velocities: (A–D) 6, 9, 13, and 7 m/sec, respectively. Voltage scale: (A, D) 20 μV; (B, C) 35 μV. Time scale: 5 msec.

review, see Beleslin, 1982). In comparison to *Myxicola*, however, values of giant fiber velocity and diameter in most polychaetes are much lower, typically in the range of 2–10 m/sec and 15–200 μm (Bullock and Horridge, 1965). As noted by Nicol (1948*b*), ratios of conduction velocity to diameter in polychaete giant fibers tend to be substantially lower than in oligochaetes, probably due to the greater degree of sheath development in the latter group.

In some sedentary polychaetes, bilaterally synchronous escape withdrawal is mediated by activity in a single medial giant fiber (e.g., *Myxicola*), but in others such responses may be mediated by spikes in paired giant fiber systems (e.g., *Eudistylia, Protula, Branchiomma*). In these paired fibers, a high probability of bilateral synchrony of spiking is maintained by "cross talk" between the fibers. The structures mediating this interaction are decussations of giant fiber neurites and various anastomotic connections, especially in anterior segments close to the normal site of giant fiber spike initiation (Bullock, 1953; Hagiwara *et al.*, 1964; Krasne, 1965). Mellon *et al.* (1980) have studied the physiological properties of the segmentally arranged commissural junctions between paired giant fibers of *Sabella* and conclude that cross talk between the fibers results from direct electrical coupling (Figure 4). However, whether the commissural junctions involve cytoplasmic fusion or gap junctions is not clear.

2.3. Efferent Pathways and Behavioral Correlates

Because there have been so few studies of the properties of giant fiber efferent pathways in polychaetes, it is difficult to make generalizations about their function. In light microscopic studies of *Nereis*, Smith (1957) and Horridge (1959) described apparent junctions between the lateral giant fibers and the axons of segmentally arranged pairs of giant motor neurons which innervate the longitudinal musculature. Electrophysiological recordings by Horridge (1959) showed that there was consistent 1:1 spike transmission between the lateral giant and giant motor axons. Initially, the giant motor neuron spike triggered a large muscle potential and longitudinal muscle twitch, but with repeated stimulation the potentials became smaller and twitches rapidly failed. With further repetitive stimulation, the 1:1 transmission at the giant–motor junction eventually failed. Subsequent to this failure, recruitment of the medial giant fiber restored firing of the motor axon. This suggests possible summation of inputs from different giant fibers systems onto the same motor neuron.

As noted by Horridge (1968), the efficacy of lateral giant to giant motor transmission is affected not only by repetitive stimulation, but also by the ongoing behavior of the animal. For example, in the absence of

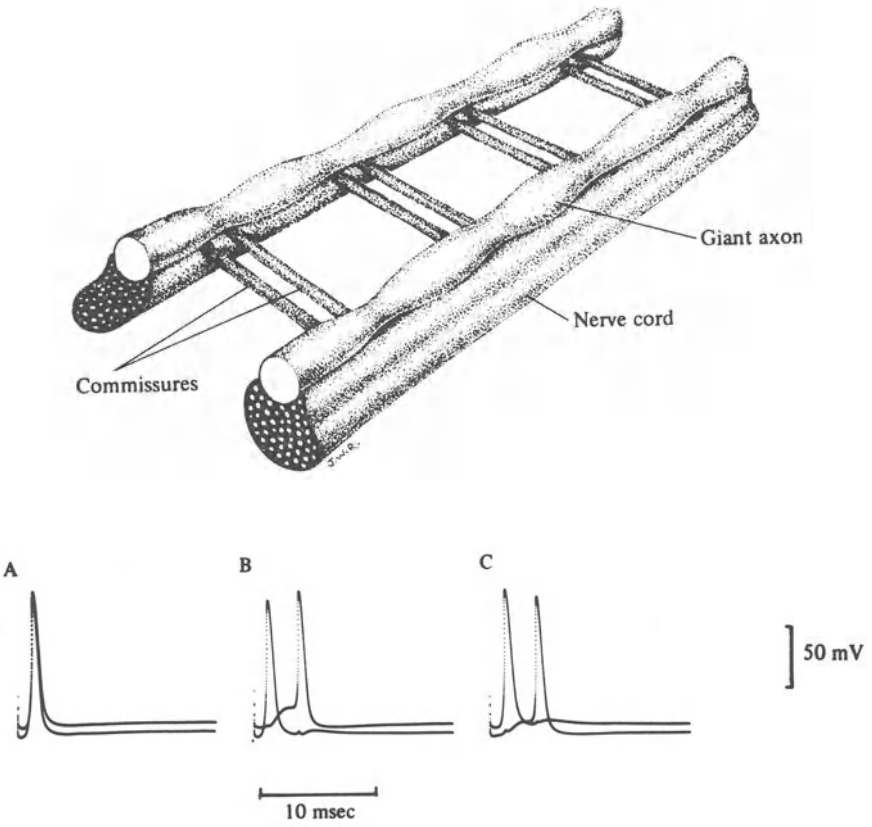


Figure 4. A diagram of the paired giant axons and lateral commissures in the nerve cord of the polychaete, *Sabella* (above). Superimposed intracellular electrical recordings from left and right giant axons of the same body segment are shown below. (A) Action potentials in the two giant axons occur synchronously in response to direct extracellular stimulation of both axons. (B) Direct stimulation of the right axon resulted in a delayed spike in the left axon. (C) Direct stimulation of the left axon resulted in a delayed spike in the right axon (from Mellon *et al.*, 1980).

crawling movements, spike transmission at the giant–motor junction was consistently 1:1, but during crawling movements lateral giant fiber spikes failed to excite the giant motor axon. These results suggest the possibility of a behavior-dependent depression of transmission at the giant–motor junction.

The timing of rapid escape movements in relation to giant fiber activity has not been extensively studied. Krasne (1965) noted that in *Branchiomma* escape withdrawal sometimes occurred without accompanying giant fiber activity, especially after repeated tactile stimulation. However,

the latency of these nongiant-mediated reactions was always greater than 40 msec, or much longer than the shortest latency of giant fiber-mediated rapid responses. Such rapid withdrawal responses occurred 10–42 msec after the first spike in a train of synaptically evoked giant fiber spikes. The withdrawal response to a single spike was submaximal, whereas with repeated spiking, responses were graded in magnitude in relation to the number of giant fiber spikes. This gradedness of escape contractions has also been noted in *Nereis* (Dorsett, 1964), but is in contrast to the situation in *Myxicola* in which a single giant fiber spike evokes a rapid and complete withdrawal of the whole animal (Nicol, 1948*a*; Roberts, 1962*c*). The average latency from this single spike until onset of rapid withdrawal was 11 msec (Roberts, 1962*c*). This value, which is several milliseconds less than the minimal latency values for earthworm escape movements (Section 4.5), appears to represent the shortest efferent response time for any annelid escape reflex. One factor that undoubtedly contributes to minimization of this time in *Myxicola* is the anatomical arrangement of the efferent pathway in which innervation of the longitudinal muscle is by peripheral axonal branches that are in cytoplasmic continuity with the giant fiber system (Nicol, 1948*c*; Wells *et al.*, 1972, 1973).

In addition to longitudinal shortening, other rapid escape movements, especially involving parapodia, have been observed. For example, in nereids, a forward pointing of the parapodia accompanied giant fiber responses (probably MGF) to stimulation of anterior segments with touch (Horridge, 1959) or decreased light intensity (Gwilliam, 1969). In contrast, backward pointing of parapodia accompanied paramedial giant fiber responses to posterior tactile stimulation (Horridge, 1959). Unfortunately, the neural control of these movements has not been studied. In summary, there is a clear need for a better understanding of the precise timing and coordination of the various neural and behavioral events involved in polychaete rapid escape responses. At least some of these determinations could be made without employing surgical invasion, since many investigators have previously reported the possibility of detecting all-or-none giant fiber spikes from the body surface in a number of polychaete species (Bullock, 1945*b*, 1948; Nicol *et al.*, 1947; Mangum and Passano, 1964; Krasne, 1965).

3. Giant Fiber Reflexes in Leeches

Light tactile stimulation of anterior segments in a leech may evoke a variety of behavioral responses including a localized withdrawal involving only a few segments. Localized withdrawal would not necessarily require mediation by a rapid, through-conducting giant fiber system, and

can be explained on the basis of the known monosynaptic connection from identified mechanoreceptive neurons (touch, pressure, and nociceptive) to segmentally arranged pairs of electrically coupled longitudinal motor neurons (Stuart, 1970; Nicholls and Purves, 1970; for review, see Muller, 1979). However, if an intense stimulus is applied to anterior segments, a rapid, symmetric shortening response involving the whole body may occur (Sawyer, 1981; Kramer, 1981; Kristan *et al.*, 1982). Such rapid responses, in which the body may shorten by as much as 70% (Sawyer, 1981), would appear to require involvement of some rapidly conducting, intersegmental pathway.

Leeches in fact do possess one relatively large fiber (Rohde's fiber = 3μm), or several such fibers, in the medial bundle (Faivre's nerve) of the ventral nerve cord (Coggeshall and Fawcett, 1964; Fernandez, 1978). In each ganglion the fiber arises from a single cell body (termed S cell; Frank *et al.*, 1975), with corresponding fibers from adjacent ganglia being electrically coupled to one another in the ganglionic connectives (Mistick, 1974; Bagnoli *et al.*, 1975*b*; Frank *et al.*, 1975). Electrical stimulation of the ventral nerve cord results in an all-or-none spike conducted in either direction along the S-cell network (or so-called "fast conducting system") at a velocity of 1.0–1.4 m/sec (Laverack, 1969; Bagnoli *et al.*, 1972, 1973). With afferent connections intact, S-cell spikes may be readily evoked by direct tactile stimulation anywhere along the animal, as well as by photic stimulation or low-amplitude water waves (Laverack, 1969; Bagnoli *et al.*, 1973, 1975*a*; Magni and Pellegrino, 1978*b*; Friesen, 1981).

Based on electrophysiological evidence, a direct excitatory connection between touch sensory neurons and S cells was proposed (Gardner-Medwin *et al.*, 1973; Bagnoli *et al.*, 1975*b*). Indeed, subsequent study by Muller and Scott (1981) confirmed that there is electrical coupling, albeit rectifying, between the touch neurons and S cells, but their morphological examination showed no areas of direct contact between processes of touch neurons and S cells (Figure 5). Rather, the cells appeared linked by a pair of small interneurons (Muller and Scott, 1981; Kramer and Goldman, 1981). The electrical junction between the touch neuron and small interneuron exhibited rectification, whereas the junction between the small interneuron and S cell was nonrectifying. The physiological significance of this arrangement is not yet clear, although one suggestion by Muller and Scott (1981) is that, depending on ongoing activity of the animal, the small connecting interneurons might function to appropriately modulate the efficacy of electrical transmission between touch neurons and S cells.

Friesen (1981) has shown that S-cell spikes are also readily evoked by stimulation of the body wall with low-amplitude water waves. Rather than activating touch neurons, these waves appear to activate a separate

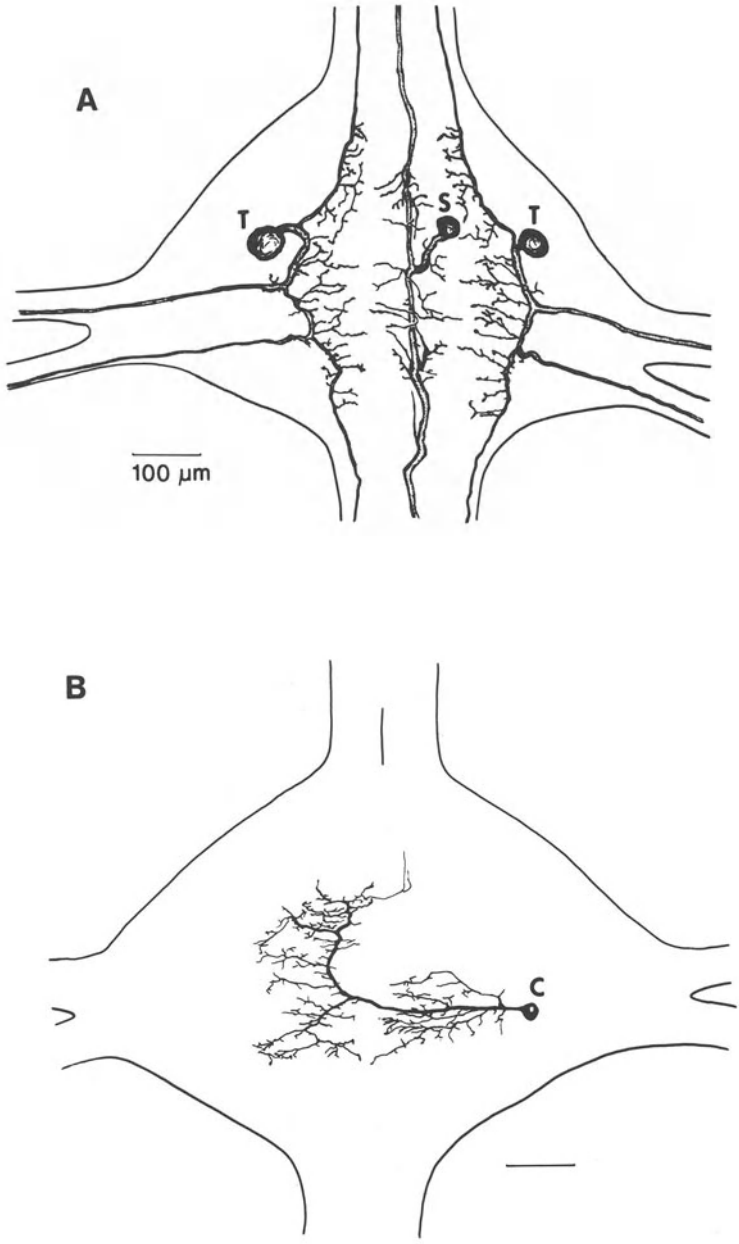


Figure 5. Morphology of touch cell, S cell, and a coupling interneuron in the ventral nerve cord of the leech, *Hirudo medicinalis*. (A) Two lateral touch cells (T) and the S cell (S) were injected with horseradish peroxidase (HRP). Note that the cells do not contact one another. (B) One of the two coupling interneurons (C) in the ganglion was injected with HRP. The processes of the interneuron span the gap between touch cell and S-cell processes (from Muller and Scott, 1981).

set of receptors, termed "sensillar movement receptors" (Friesen, 1981). Intracellular recordings of S-cell responses to wave stimulation indicate that spike initiation may require summation of movement receptor inputs. However, the details of the synaptic connections in this pathway and possible behavioral consequences of S-cell activation under these conditions have not been investigated.

The S-cell responses to photic stimulation are probably mediated by two different photosensory structures: five bilateral pairs of eyes on the dorsal surface of the head and seven bilateral pairs of sensilla on each of the 21 body segments (Kretz *et al.*, 1976). The rapid adaptation that characterizes S-cell responses to repeated photic stimulation (Laverack, 1969; Bagnoli *et al.*, 1973) is probably not attributable either to peripheral accomodation of the photoreceptors or to depressed excitability of the S cells (Kretz *et al.*, 1976). Since connections from photoreceptors to S cells are probably not monosynaptic (Bagnoli *et al.*, 1975*a*), failure of S-cell responses to repeated photic stimulation possibly could involve several sites along the afferent pathway.

There is some evidence for interaction between the converging mechanoreceptive and photoreceptive pathways. Bagnoli *et al.* (1973) found a suppression of S-cell responses to photic stimulation when this stimulation occurred within 120 msec after mechanical stimulation. However, the responses to photic stimulation were augmented if they occurred approximately 500–700 msec after mechanical stimulation. Chronic recordings from the ventral nerve cord of unrestrained leeches indicate that S-cell spiking does not occur during normal creeping, swimming, or respiratory movements, thus suggesting an absence of any reafferent excitation of S cells during these activities (Magni and Pellegrino, 1978*b*).

A recent study by Belardetti *et al.* (1982) has demonstrated another important sensory modality interaction in the S-cell system, namely between nociceptive and touch receptor inputs to the S cell. For example, if repeated mechanical stimulation is applied to the body wall, a rapidly developing and prolonged decrease in the number of S-cell spikes with each stimulus is observed. However, strong nociceptive stimulation to head segments can readily restore, or potentiate, the S-cell response to touch. Several kinds of experiments by Belardetti *et al.* (1982) demonstrate that serotonin and cyclic AMP (cAMP) may be involved in this potentiation effect. These include experiments showing that (1) serotonin application can potentiate previously decremented responses to either tactile stimulation of the body wall or electrical stimulation of segmental roots, (2) methysergide, an antagonist of serotonin, can block serotonin-induced potentiation of the S-cell response, (3) imidazole, a cAMP phosphodiesterase activator, can abolish the potentiation of S-cell firing by

serotonin, and (4) application of db-cAMP, a cAMP analog, can potentiate the S-cell response to electrical stimulation of ganglionic roots.

The efferent connections of S cells include excitatory inputs onto motor neurons innervating annulus erector muscles and the so-called L-motor neurons innervating the longitudinal musculature (Gardner-Medwin *et al.*, 1973); however, only the S cell-to-L motor neuron pathway has been studied in any detail. Intracellular recordings have shown that the L cells receive excitatory input from the S cell (Magni and Pellegrino, 1978*a*; Kramer, 1981) and that the excitation probably involves an electrotonic junction (Gardner-Medwin *et al.*, 1973; Magni and Pellegrino, 1978*a*). Although Magni and Pellegrino (1978*a*) have shown that a single S-cell spike can frequently elicit a suprathreshold PSP (postsynaptic potential) in the L-motor neuron, detectable shortening does not usually accompany a single L-motor neuron spike (Stuart, 1970). The prerequisite for observable shortening appears to be a train of S-cell spikes. In dissected preparations of *Haementeria ghillianii,* intracellular stimulation of S cells at frequencies of 45 spikes/sec elicited shortening responses (Kramer, 1981). In contrast, in intact *Hirudo medicinalis,* a train of S-cell spikes at 60 spikes/sec (evoked by photic stimulation) was not accompanied by a clear-cut shortening response. However, higher frequency bursts of S-cell spiking in *Hirudo* (evoked by tactile stimulation) were accompanied by observable shortening (Magni and Pellegrino, 1978*a*). Taken together, these results seem to indicate that there is some critical number and frequency of both S-cell and L-motor neuron spikes required for evoking a just detectable shortening response; however, there may be important species differences in these critical values as well as alterations of these values due to dissection procedures. In any individual animal, gradations in the amount of shortening would occur as the number of frequency of S-cell and L-motor neuron spiking exceeded these critical values.

In addition to S-cell involvement in activating the body wall longitudinal musculature, Magni and Pellegrino (1975) have shown that repetitive S-cell firing is accompanied by a generalized contraction of longitudinal muscle fibers in the ventral nerve cord sheath. Subsequent study has shown that body wall and nerve cord shortening responses are mediated by the same set of segmentally arranged longitudinal motor neurons (Magni and Pellegrino, 1978*a*).

In summary, there is evidence that leeches possess a relatively large caliber, through-conducting pathway whose organization is reminiscent of the giant fiber systems of other annelids and whose functions include integration of inputs from a variety of stimulus modalities and subsequent mediation of a widespread reflexive shortening of the animal. Some par-

ticularly interesting topics for future study include further examination of the cellular basis of sensory modality interactions and plasticity of function in the shortening reflex, as well as study of the possible intrinsic modulation of reflex sensitivity in relation to ongoing behavior.

4. Rapid Escape Reflexes in Oligochaete Earthworms

Giant nerve fibers are found in the ventral nerve cords of many fresh water oligochaetes and nearly all earthworms (Stephenson, 1930; Prosser, 1934; Bullock and Horridge, 1965; Jamieson, 1981). However, it is only in the latter group that there have been any correlated anatomical, physiological, and behavioral studies relating to rapid escape reflexes. In this section some of the important anatomical specializations and physiological properties that underlie rapid escape reflexes of eathworms will be reviewed. Except when noted, discussion of the neuronal circuitry underlying earthworm escape reflexes will refer to the most extensively studied species, *Lumbricus terrestris* (Family Lumbricidae). Although detailed studies of escape reflex circuitry have not been made in most other earthworm families, we may infer from electrophysiological records, such as those in Figures 2 and 3, that there may be numerous common features of escape reflex design and function in most if not all earthworm families (Drewes *et al.*, 1983).

4.1. Anatomical Organization of Giant Fibers

Most earthworms possess three prominent giant nerve fibers: one medial fiber (MGF) and two lateral fibers (LGF). These are located in the dorsal portion of the ventral nerve cord (Figures 6 and 7). In *Lumbricus terrestris,* Günther (1971*b*) has also described a pair of smaller "ventral giant fibers" in the ventral portion of the cord, which appear to be organized as chains of segmentally arranged interneurons. The physiological role of the ventral giant fibers is unknown, although Günther noted that numerous collaterals of the ventral giants extend into the ventral longitudinal sensory bundles. In contrast to the dorsal giant fibers, there is no evidence that the ventral giants form a multisegmental through-conducting pathway.

In cross section, the MGF and LGF are easily identified by their large diameter and the presence of a well-developed myelinlike sheath that surrounds each fiber (for histological and ultrastructural descriptions of the sheath, see Taylor, 1940; De Robertis and Bennett, 1956; Hama, 1959; and Günther, 1976). In each segment Günther and Walther (1971)

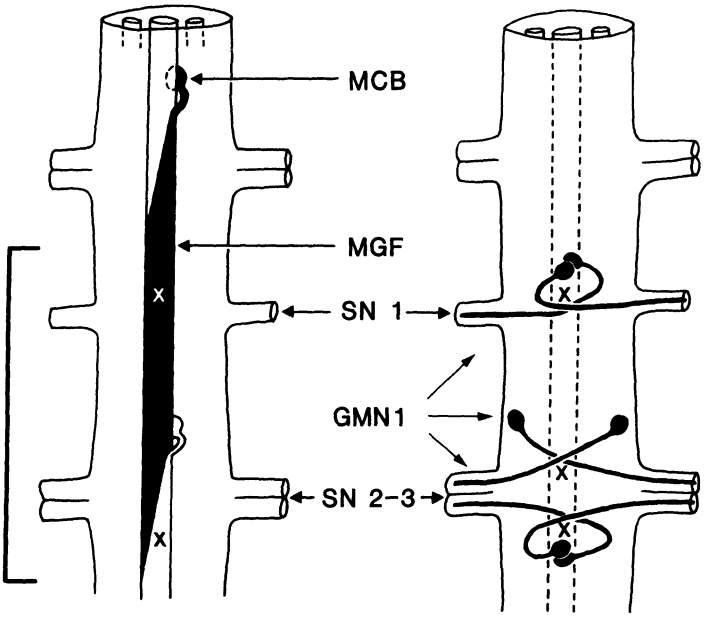


Figure 6. Diagram of general cellular organization of MGF and its associated giant motor neurons in the earthworm ventral nerve cord. (A) The medial giant cell body (MCB) and its neurite are shown displaced slightly to the right of the MGF proper. In each segment of the ventral nerve cord (bracket), three ventrally projecting MGF collaterals are found, their approximate positions being indicated by the two X's and by the neurite of the medial giant cell body. (B) Three pairs of giant motor neuron (GMN1) cell bodies are shown. The GMN1 axons decussate at the sites of the three MGF collaterals (X's), and pass out each of the segmental nerves (SN 1 and 2–3). See Section 4 for further details. (These diagrams are based on anatomical descriptions by Mulloney, 1970; Günther, 1971*a*, 1972; and Günther and Walther, 1971.)

noted that the sheath is interrupted ventrally by collateral projections arising from the MGF and LGF, the number and position of these collaterals being constant from segment to segment. Three ventral collaterals are associated with the MGF, two of which are relatively short and terminate in the central neuropil of the central nerve cord at the levels of the first and third segmental nerves. The remaining MGF collateral is longer and actually represents the neurite of the MGF cell body, which is located ventromedially in the nerve cord (Mulloney, 1970; Günther, 1971*a*; Günther and Walther, 1971; Schürmann and Günther, 1973).

Collectively, the MGF collaterals represent the sites for synaptic inputs and outputs (Günther and Walther, 1971; Günther and Schürmann, 1973). The main outputs of the MGF, to be discussed in Section 4.4,

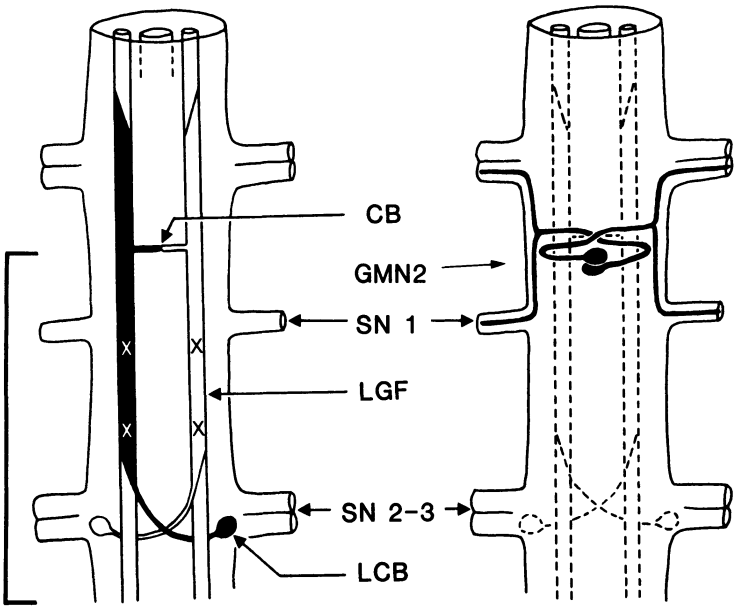


Figure 7. Diagram of the general cellular organization of the earthworm LGF and its associated giant motor neurons in the ventral nerve cord. (A) In each segment of the cord (bracket), the LGF system is derived from a pair of lateral giant cell bodies (LCB). The neurites of these cell bodies decussate and enlarge into the LGF proper. The four LGF collaterals in each segment include the lateral cross-bridge (CB), two ventral collaterals (X's), and the neurite of the lateral giant cell body. (B) The pair of giant motor neurons (GMN2) whose axons decussate and are closely associated with the LGF cross-bridge. Each GMN2 axon bifurcates and passes out segmental nerves 1 and 3 of adjacent segments. (These diagrams are based on anatomical descriptions by Mulloney, 1970; Günther, 1971*a*, 1972; and Günther and Walther, 1971.)

appear to be three pairs of giant motor neurons in each segment (Figure 6). With regard to possible afferent inputs, none of the three MGF collaterals appear to be directly associated with identifiable longitudinal sensory bundles in the ventral neuropil of the nerve cord. Rather, the association appears to be indirect, involving an interposed "giant interneuron," whose collaterals are associated with the ventral sensory neuropil and with the three MGF collaterals. The diameter of the paired, segmentally arranged giant interneurons, as well as the caliber and length of their collaterals, are greatest in anterior segments and steadily decrease in more posterior segments. Since these morphological gradients correlate with physiological evidence for anterior localization of the MGF sensory field, Günther and Walther (1971) have suggested that the giant interneurons function as afferent processing interneurons. Ultrastructural study of the

junction between the giant interneuron and MGF suggests a possible electrical synapse (Günther and Schürmann, 1973).

Four identifiable collaterals project ventrally from each LGF in a segment. One of these collaterals, after branching, becomes the neurite of the LGF cell body, which is located ventrolaterally on the opposite side of the nerve cord (Günther, 1971*a*; Günther and Walther, 1971; Schürmann and Günther, 1973). Neurites arising from the paired LGF cell bodies in each segment usually come into close association with one another at a point of decussation ventral to the MGF (Mulloney, 1970; Günther, 1971*a*; Günther and Schürmann, 1973). Another of the four LGF collaterals projects somewhat medially where one of its branches forms a cross-bridge with the corresponding branch of the contralateral LGF. Thus there are two sites of close anatomical association between left and right LGFs in each segment and these involve branches from two different collaterals in each LGF. These collateral connections, in particular the cross-bridges (Mulloney, 1970), between left and right LGFs are undoubtedly the basis both for the observed electrotonic coupling between the two LGFs (Wilson, 1961) and the resultant bilateral synchronization of LGF spikes during propagation along the nerve cord (Rushton, 1945*a*).

Collectively, the LGF collaterals, as well as dendritic branches arising from the LGF cell bodies, represent the sites of synaptic inputs and outputs for the LGF (Günther and Walther, 1971; Günther and Schürmann, 1973). The outputs of the LGF system, to be discussed in a later section, include a pair of giant motor neurons in each segment (Figure 7). In regard to possible inputs, Günther and Walther (1971) noted that in posterior segments the LGF collaterals were relatively long and thick and projected into the ventral sensory neuropil. This suggests that afferent-to-LGF connections, at least in posterior segments, may be direct. However, apparent inputs onto the LGF from other sources, including the giant interneurons, were also noted (Günther and Walther, 1971; Günther and Schürmann, 1973). The mutual association of both LGF and MGF systems with the giant interneurons raises questions regarding possible MGF to LGF interactions, but there is yet no physiological evidence that spiking in one giant fiber system modifies in any way the properties of the other giant fiber system.

The anatomical associations between giant fiber cell membranes from adjacent segments typically consist of obliquely arranged partitions, or septa (Stough, 1926). There have been numerous ultrastructural studies describing these intersegmental membrane appositions and associated specializations in the MGF and LGF (Hama, 1959; Dewey and Barr, 1964; Coggeshall, 1965; Oesterle and Barth, 1973, 1981; Günther, 1975; Kensler *et al.*, 1979). In particular, the study by Kensler *et al.* (1979) using thin-

section and freeze-fracture techniques has clearly demonstrated the presence of gap junctions between the LGFs of adjacent segments. Some demonstrated physiological properties that correspond with these ultrastructural observations include low transseptal resistance, nonrectification, and permeability of septa to small molecular weight dyes (Brink and Barr, 1977; Brink and Dewey, 1978, 1980; Kensler *et al.*, 1979). The nonrectifying properties of the septum account for the capability of bidirectional impulse conduction that was originally demonstrated in several classic studies of conduction in earthworm giant fibers (Eccles *et al.*, 1933; Bullock, 1945*a*; Rushton, 1945*a*).

4.2. Conduction Properties of Giant Fibers

In animals with highly elongated bodies, there can be a substantial saving in escape reflex time by maximization of giant fiber conduction velocity along the animal (for discussions, see Bullock, 1952; Kennedy, 1966; and Bennett, 1977). A number of expected, as well as unusual factors, contribute to the relatively high conduction velocities of earthworm giant fibers. These include: large diameter, influence of the sheath, formation of syncytia, and facilitation of conduction velocity.

Perhaps the most obvious factor contributing to rapid conduction is fiber diameter. In earthworm giant fibers the relationship between velocity and diameter appears to be linear over a wide range of fiber diameters (Adey, 1951; O'Gara *et al.*, 1982). The slope of the relationship is about 0.5; that is, a 10 m/sec increase in velocity for each 20 μm increase in diameter. This value is remarkably greater than for most other invertebrate giant fibers (cf. Table 3.4 in Bullock and Horridge, 1965). For example, in the giant axon of the squid stellar nerve, for which a linear velocity–diameter relationship was also found, the slope was only 0.065 (Hodes, 1953). The substantially higher value in the earthworm appears related, at least in part, to the presence of the myelinlike sheath that surrounds each giant fiber.

The sheaths of both MGF and LGF in earthworms are interrupted in each segment by ventrally projecting collaterals. However, in the MGF there are additional openings in the dorsal surface of the sheath (Günther, 1973*a*, 1976). These consist of two "nodes" (10–15 μm in diameter) in each segment. Günther's measurements of longitudinal currents along the surface of the MGF indicated the nodes act as current sources and sinks during action potential propagation. His records of electrically evoked spikes along the fiber indicated a step-wise increase in latency of 40–90 μsec at each node. Also, when a direct electrical stimulus was applied, the threshold current for an MGF spike at the internodal region was an

order of magnitude greater than with stimulation at the nodal region. Based on these observations, Günther concluded that the dorsal nodes function as focal points for electrogenesis, and that there is a saltatory-type impulse conduction in the earthworm MGF.

Aside from giant fiber diameter and sheath influences, another factor that contributes to the minimization of conduction time is the formation of multineuronal syncytia by elimination of septal boundaries in the giant fibers (Günther, 1971*a*, 1975). Günther (1971*a*) found that about 60% of the septa were missing in the MGF and 20% in the LGF. One apparent consequence of septal elimination, as determined by Brink and Dewey (1980), is that conduction velocity across a septum is much slower (8 m/sec) than the velocity in the same fiber in the absence of a septum (25 m/sec). Assuming a conduction distance of 400 μm for both of these values and a uniform velocity of 25 m/sec along the fiber except across the septum, then the difference in conduction time (34 μsec) is an estimate of the actual delay time across the septum. This delay time, when multiplied by the number of missing septa in a worm (60% of MGF septa in a 150 segment worm), gives an overall time savings of more than 3 msec, which is a substantial percentage of the total MGF conduction time along the animal (usually about 5 msec).

Another factor that affects spike conduction velocity in earthworm giant fibers is antecedent spiking activity in the fibers. Bullock (1951) discovered that in the isolated ventral nerve cord of the earthworm, *Lumbricus terrestris* (as well as in nerve fibers of a polychaete, shrimp, and frog), giant fiber conduction velocity increased by as much as 10–20% for spikes occurring shortly after a "conditioning" spike. This phenomenon, referred to by Bullock as "facilitation of conduction velocity," was most pronounced about 6 msec after the conditioning spike, but was still evident at intervals greater than 100 msec. The same phenomenon, involving velocity increases of 20%, has been observed in recordings of naturally evoked MGF and LGF spikes in intact earthworms, *Lumbricus* and *Eisenia* (Drewes *et al.*, 1978; O'Gara *et al.*, 1982). One result of the phenomenon, as shown in Figure 8, is that the interspike interval between a given pair of spikes may gradually diminish as spikes are conducted along the animal. A physiological consequence of the reduced interspike interval, as discussed in a later section, is the increased probability for a pair of LGF spikes to trigger a giant motor neuron spike in anterior segments, where LGF interspike intervals are shortest. The biophysical mechanism that permits conduction at a supernormal velocity is unknown. However, as noted by Bullock (1951), the mechanism is probably unrelated to the presence of septa, since supernormal conduction velocity was also seen in a nonseptate polychaete giant fiber.

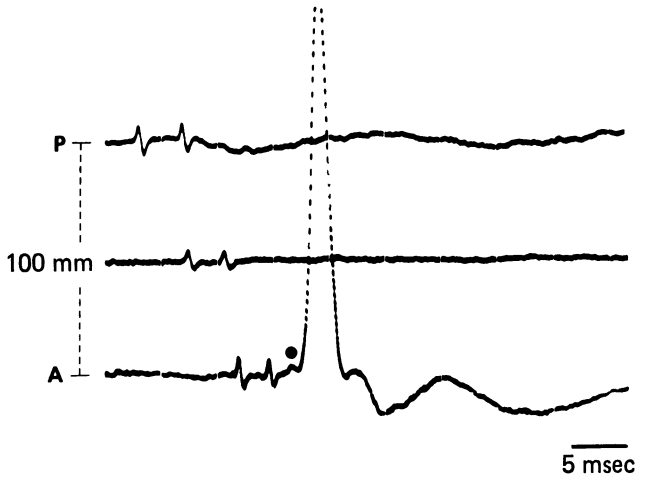


Figure 8. Reduction of interspike interval by facilitation of LGF conduction velocity in *Lumbricus terrestris*. A pair of LGF spikes, initiated by light tactile stimulation of the tail, was detected at three different recording sites along the intact animal: upper trace (segment 135), middle trace (segment 78), and lower trace (segment 33). The LGF interspike interval for the two spikes near the posterior end (P) was approximately 4 msec. The average conduction velocity of the first spike was about 11 m/sec and that of the second about 13 m/sec. Thus at the most anterior recording site (A), the interspike interval was reduced to less than 3 msec. At the anterior recording site the second LGF spike was followed by a GMN2 spike (•) and large longitudinal muscle potential (from Drewes and McFall, 1980).

Based on measurements from intact, unanesthetized worms (adult *Lumbricus terrestris*), typical values of normal, nonfacilitated conduction velocity are 30–38 m/sec for the MGF and 11–15 m/sec for the LGF (McFall *et al.*, 1977). Since these values are considerably higher than values from isolated cords (cf. Kao and Grundfest, 1957; Goldman, 1963, 1964; Lagerspetz and Talo, 1967), it appears that isolation procedures somehow adversely affect spike conduction properties. It is not yet clear to what degree such procedures might alter functioning of other escape reflex components such as synapses. Considering the marked lability of the reflex even in the intact animal, other substantial effects, either quantitative or qualitative in nature, are likely to accompany isolation procedures.

4.3. Afferent Pathways

In earthworms the MGF and LGF can be activated by a variety of abruptly delivered mechanical stimuli including body wall touch or pressure and substrate vibration (Bullock, 1945*a*; Adey, 1951; Morita and

Tateda, 1952; Günther, 1973*b*; Drewes *et al.*, 1978; Moore, 1979; Smith and Mittenthal, 1980). Unlike the giant fiber reflexes in polychaetes and leeches, there is not yet any experimental evidence for the adequacy of photic stimuli in evoking giant fiber spikes in earthworms, at least under laboratory conditions. However, a common field observation by those who nocturnally collect earthworms, such as *Lumbricus terrestris,* is that withdrawal responses of varying speeds are often evoked by abrupt onset of intense light. One question that remains unanswered is whether withdrawal responses to photic stimulation, under any circumstances, are giant fiber mediated. If so, are giant fibers excited directly via some photosensory to giant fiber pathway or, alternatively, could excitation involve an initial nongiant-mediated withdrawal followed by reafferent excitation of mechanosensory to giant fiber pathways, as suggested in some polychaetes (Gwilliam, 1969)? Failure of photic stimulation to evoke giant fiber spikes under any circumstances, of course, does not preclude the possibility that photic stimulation produces a subthreshold biasing of giant fibers toward spiking.

Functional distinctions between MGF and LGF afferent pathways are readily demonstrated by mapping of their respective sensory fields. Tactile stimulation of the most anterior segments invariably evokes MGF spikes, whereas stimulation of the most posterior segments invariably evokes LGF spikes (Figures 2 and 3). In *Lumbricus terrestris* the sensitivities of the MGF and LGF fields are especially high at the ends of the animals and diminish markedly toward middle segments (Günther, 1973*b*). Usually there is an area of substantial overlap between the two fields, this area being centered about one third of the way back along the animal (Günther, 1973*b*; Moore, 1979). The exact amount of overlap varies depending on factors such as interspecific differences, intraspecific variation, the nature of the stimulus, and the amount of previous stimulation.

At least some of the sensory neurons involved in giant fiber excitation have been identified and consist of serially homologous mechanosensory neurons with cell bodies in the ventral nerve cord and with axons projecting into segmental nerves. Based on anatomical criteria, these neurons have been classified into two groups (Günther, 1970, 1971*b*). One group, termed PN2, consists of five to seven bilaterally arranged pairs of neurons per segment. Axons from the PN2 neurons pass longitudinally through the third segmental nerve. The other group PN3, consists of two pairs of neurons, each neuron giving rise to two (or possibly three) axonal branches that exit ipsilaterally through the first and third segmental nerves of the same segment or through the first and third segmental nerves of adjacent segments.

Physiological differences have been demonstrated between the PN2

and PN3 groups (Günther, 1970; Smith and Mittenthal, 1980). Extracellular recordings from individual segmental nerves indicate that light tactile stimulation of the body wall (with a force greater than 3 *g*) evokes a phasic burst of spikes in which one or two large amplitude neural units can be readily distinguished. Simultaneous recordings from neighboring segmental nerves indicate that these spikes enter the cord through one segmental nerve and exit the cord via a neighboring ipsilateral segmental nerve (Figure 9). The large spike amplitudes, as well as the spatial and temporal distribution of these spikes in segmental nerves, indicate the spikes arise from the large-diameter, branched axons of the PN3 sensory neurons. Based on their responsiveness to relatively light mechanical stimulation, the PN3 neurons have been physiologically classified as touch-sensitive neurons (Günther, 1970; Smith and Mittenthal, 1980).

Physiological criteria for identification of PN2 spiking are also consistent with anatomical features (Günther, 1970; Smith and Mittenthal,

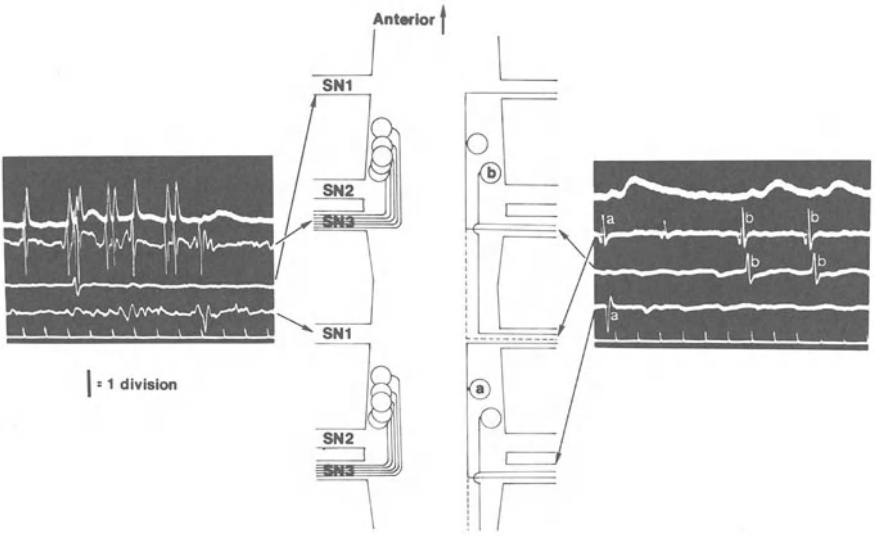


Figure 9. Afferent spiking and associated postsynaptic potential (PSP) activity in the LGF of *Lumbricus terrestris*. Two segments of ventral nerve cord, with the cell bodies and processes of touch neurons shown in the right half of the cord and those of pressure neurons shown in the left half. (Right) Recordings show typical responses of touch neurons from SN1 (intact) and two adjacent SN3s (cut). The top trace shows the associated PSPs in the LGF. Spikes labeled "a" derive from processes of cell "a" in the diagram; spikes labeled "b" derive from processes of cell "b." (Left) Recordings show typical responses of pressure neurons from SN3 (intact) and subsequent PSPs in the LGF (top trace). Time mark = 10 msec/div. One division = 2 mV for intracellular traces and 500 μV for extracellular records from segmental nerves (from Smith and Mittenthal, 1980).

1980). Extracellular electrical records from the third segmental nerve indicate that PN2 spikes are evoked by tactile stimulation of the body wall and consist of relatively large amplitude spikes from several different units (Figure 9). Unlike the responsiveness of PN3 neurons, PN2 spiking activity is tonic and requires relatively strong tactile stimulation (forces from 10–20 *g*). Based on these response patterns, the PN2 neurons have been classified as pressure-sensitive neurons.

The synaptic inputs from touch- and pressure-sensitive neurons onto MGF and LGF have been studied by Smith and Mittenthal (1980). In both MGF and LGF each touch neuron spike evokes a small depolarizing PSP that peaks after the touch neuron spike enters the ventral nerve cord. In some cases this short latency PSP is accompanied by a later and a slower PSP of more variable waveform. As shown in Figure 10, the amplitude of the early PSP in the MGF is greatest in anterior segments and absent in posterior segments. Conversely, in the LGF, the PSP is largest in tail segments and barely detectable in anterior segments. In middle segments both MGF and LGF receive inputs from touch neurons.

Synaptic inputs from pressure neurons onto MGF and LGF consist of unitary depolarizing PSPs, each being initiated approximately 3 msec after a pressure neuron spike. The constancy of PSP amplitude and the

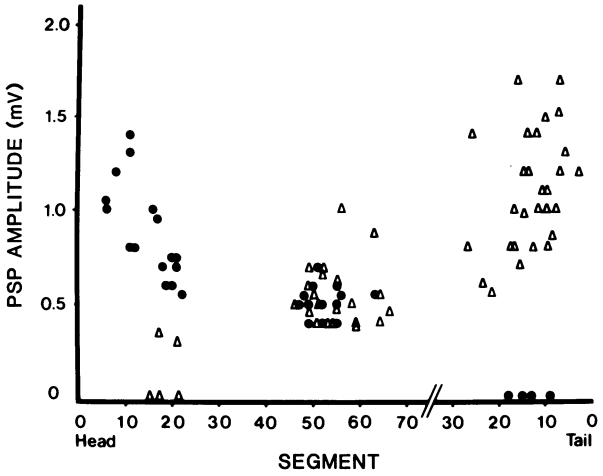


Figure 10. Amplitudes of early phase of giant fiber PSPs generated by touch neurons in *Lumbricus terrestris*. The ordinate indicates the PSP amplitude in mV (see Figure 9). Numbers before the break in the abscissa indicate the number of segments away from the head; numbers after the break indicate the number of segments away from the tail. Solid circles show values for MGF PSPs and triangles for LGF PSPs (from Smith and Mittenthal, 1980).

absence of PSP failures during high frequency bursts of pressure neuron spikes are suggestive of an electrical synapse between afferent and giant nerve fibers. Longitudinal gradients in PSP amplitude for pressure neuron inputs closely parallel those seen in Figure 10 for touch neuron inputs. The monotonic longitudinal gradients in the strength of the synaptic connections would appear to provide a physiological basis for the spatial arrangements and sensitivity gradients of the MGF and LGF sensory fields as determined by Günther (1973*b*). However, there is the possibility that other, as yet unidentified, mechanoreceptors may excite the giant fibers (Smith and Mittenthal, 1980), and perhaps these contribute to the observed gradients in giant fiber sensory fields. In addition, possibilities for segmental variations in the locus or threshold of giant fiber spike initiation have not been eliminated.

One important consideration in Smith and Mittenthal's study of the afferent to giant fiber pathway is the relatively small amplitude of giant fiber PSPs evoked by touch and pressure neuron spiking activity and the usual failure of high-frequency trains of PSPs to evoke giant fiber spikes in their dissected preparations. These observations are not easily reconciled with results from intact animals that show that giant fiber spikes are readily initiated by focal mechanical stimulation, with latencies from stimulus onset to giant fiber spikes being as short as 5 msec (Figure 3) (see also Günther, 1970; Moore, 1979). This amount of time is sufficient to allow generation of no more than one or perhaps two spikes in any given afferent neuron. Possible reasons for failure of PSP trains to evoke giant fiber spikes in dissected preparations might be a general depression of afferent to giant transmission or reduction in excitability of the giant fibers due to surgical trauma.

In view of the segmental repetition of afferent to giant connections, future studies of the intersegmental integration of afferent inputs would appear especially apropos. Intersegmental integration of inputs may be particularly important during stimulus conditions such as substrate vibration, which could result in nearly synchronous activation and spatial summation of afferent inputs from many different segments. Considering the relatively long space constant of the giant fibers (approximately 3–6 mm) (Goldman, 1964; Dierolf and Brink, 1973), spatial summation of inputs from points as far as 5–10 segments apart would be expected. Also, one would expect giant fibers to be particularly responsive to multisegmental inputs and less responsive to unisegmental input. This is because synaptic input currents occurring at a single locus would be more diffusely shunted throughout the giant fiber network. As a consequence of this shunting (loading), PSP amplitude would be reduced due to an effectively

low input resistance and short time constant (Berry and Pentreath, 1977; Dorsett, 1980). In contrast, with synchronous inputs at several loci, there would be less loading of synaptic input currents because little or no voltage difference would exist at the points of input along the giant fiber. Consequently, the effective input resistance and time constant would be relatively great, making PSPs larger and longer.

4.4. Efferent Pathways

As shown in Figure 6, the MGF system is closely associated with three pairs of serially homologous giant motor neurons, termed GMN1 (Günther and Walther, 1971; Günther, 1972). In the middle of the nerve cord, each GMN1 axon makes contact with an MGF collateral and with the corresponding contralateral GMN1 axon at the point of axonal decussation. These connections undoubtedly help to assure bilateral synchrony in the excitation of GMN1 axons. Diameters of the GMN1 axons are 15 μm in the first and third segmental nerves and 9 μm in the second nerve. Corresponding to these relatively large diameters, extracellular spikes in GMN1 axons are distinguishable by their large amplitudes (1.5–3.0 mV) and rapid conduction velocities (1.25–1.85 m/sec) (Günther, 1972).

Electrophysiological studies of dissected as well as intact animals have shown that each MGF spike is coupled in 1:1 fashion to a GMN1 spike (Günther, 1972; Drewes *et al.*, 1980). The short time lag from the MGF spike in the cord until the GMN1 spike in segmental nerves (minimum = 0.6 msec), as well as the consistent 1:1 coupling with repetitive stimulation, suggest that an electrical junction is involved (Günther, 1972). If the MGF is electrically coupled to all three GMN1 pairs in each segment, then the sequential excitation of GMN1 axons during spike propagation along the animal may impose a substantial shunting or loading of MGF spike currents. The large diameter of the MGF and the resultant large spike currents may help to offset this tendency (Dorsett, 1980).

Descriptions of the innervation patterns of GMN1 axons along the animal are only partially complete. In anterior segments at least some GMN1 axons appear to innervate the longitudinal musculature, as indicated by the negative-going longitudinal muscle potentials that accompany GMN1 spikes (Günther, 1972); however, the possibility of some GMN1 axons innervating other muscle units, such as septal muscles, has not been eliminated. In tail segments of *Lumbricus terrestris,* Pallas and Drewes (1981) have shown that each MFG spike is coupled in 1:1 fashion with a large amplitude spike in a motor axon that appears to innervate the septal musculature. It is not yet clear whether these motor axons arise from a

uniquely derived set of motor neurons or from motor neurons that are serial homologues of the GMN1 neurons innervating longitudinal muscle in other segments.

With repetitive stimulation in dissected preparations, GMN1-mediated muscle potentials show marked facilitation in amplitude (Günther, 1972). Likewise, in noninvasive recordings from the ventral surface of intact animals, facilitation of these potentials is clearly evident (Drewes *et al.*, 1980). Such recordings also show there is a pronounced longitudinal gradient in the amount of facilitation along the animal, the greatest amount occurring in the most anterior 60 segments and gradually less in more posterior segments (Figure 11). One hypothesis that would explain these observations is the occurrence of a longitudinal gradient in the tendency for facilitated transmitter release at GMN1 terminals along the animal.

As shown in Figure 7, a separate set of serially homologous giant motor neurons (termed GMN2) is associated with the LGF system. Each GMN2 axon makes contact both with the LGF system, at its collateral cross-bridge, and with the corresponding contralateral GMN2 axon, at the point of axonal decussation. The GMN2 axon then bifurcates, a rostral branch exiting the nerve cord via the third segmental nerve and a caudal branch exiting via the first segmental nerve of the next posterior segment. The diameter of GMN2 axons in the segmental nerves is about 9 μm. Corresponding to this relatively large diameter, spikes in GMN2 axons are identifiable in extracellular records by their large amplitude (about 1.5 mV) and rapid conduction velocity (1.25 m/sec) (Günther, 1972).

The transmission between LGF and GMN2 appears less reliable than between MGF and GMN1. In dissected preparations, the first LGF spike in a train does not trigger a GMN2 spike, but each LGF spike thereafter successfully triggers a GMN2 spike. The time lag between LGF and GMN2 spikes is somewhat variable, ranging from a minimum of 0.9 msec to more than 2.0 msec (Günther, 1972). The exact nature of the transmission process between LGF and GMN2 is unknown, but the close anatomical association of LGF and GMN2 is consistent with some type of monosynaptic transmission (Günther and Walther, 1971).

The peripheral connections of GMN2 axons appear to involve the longitudinal musculature in all segments. In dissected animals each GMN2 spike evokes a relatively large, negative-going longitudinal muscle potential that decreases in amplitude, rather than facilitates, during repeated stimulation (Günther, 1972). The initially large amplitude and subsequent antifacilitation of GMN2-mediated muscle potentials correspond to properties of the so-called "fast-axon"-mediated longitudinal muscle potentials evoked by direct segmental nerve stimulation, as described by Drewes and Pax (1974). The initially rapid antifacilitation of these potentials may

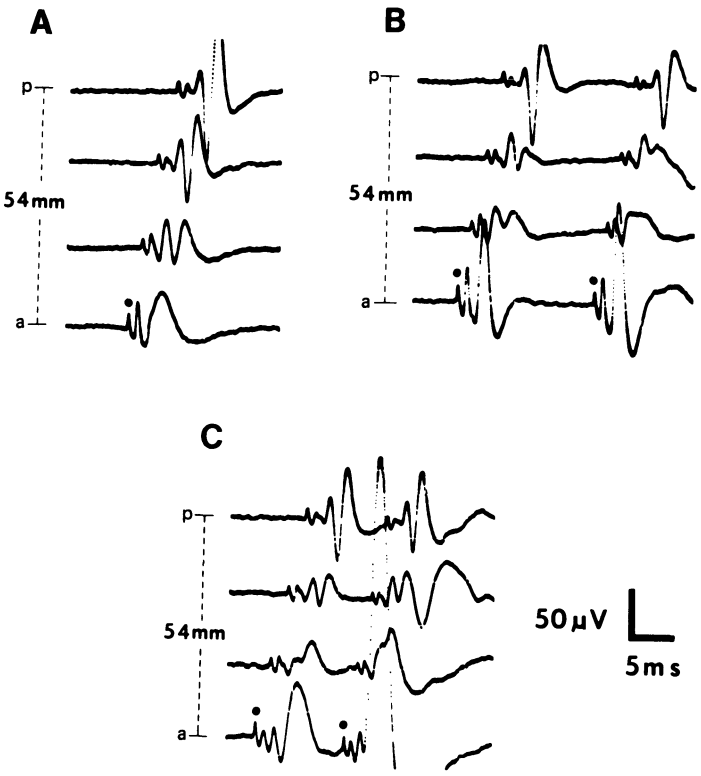


Figure 11. MGF activity in an adult *Eisenia foetida* recorded simultaneously at four sites along the intact animal. The most anterior recording site (a) (approximately segment 20) was 54 mm from the most posterior site (p) (approximately segment 85). The distance between adjacent recording sites was 18 mm. (A) The single MGF spike (•) was evoked by very light tactile stimulation of the head. The spike was conducted posteriorly at a velocity of 10 m/sec. At each recording site the MGF spike was followed after approximately 1.5 msec by an apparent motor neuron spike and longitudinal muscle potential. (B) The muscle potentials accompanying two widely spaced (interspike interval, 16 msec) MGF spikes (•) showed little or no facilitation in amplitude. (C) The muscle potentials accompanying two closely spaced (interspike interval 10 msec) MGF spikes (•) showed marked facilitation, especially in the most anterior record (from O'Gara *et al.*, 1982).

be attributed to some type of synaptic depression at the GMN2 neuromuscular junctions.

The potentials generated by LGF and GMN2 spiking are sufficiently large to permit detection with electrodes in contact with the ventral surface of intact animals (Figure 8). Such *in vivo* recordings have shown that the effectiveness of LGF to GMN2 transmission is not uniform along the

animal. The transmission is most effective in anterior segments where a single LGF spike occasionally triggers a GMN2 spike and a pair of closely spaced LGF spikes (e.g., 4–6 msec interspike interval) is nearly always effective in triggering a GMN2 spike. The efficacy of transmission diminishes in a monotonic anterior-posterior gradient along the animal, except in extremely posterior segments where there is an increase in efficacy (Drewes and McFall, 1980).

Besides the longitudinal gradient, another factor that influences the likelihood of GMN2 activation in a segment is the interspike interval between a pair of LGF spikes. For example, a pair of LGF spikes with an interspike interval of 2–4 msec effectively triggers a GMN2 spike in more than 80% of the tests in segments 31–60. However, if interspike intervals are 12–14 msec, GMN2 spikes are triggered in only about 5% of the tests from the same region (Drewes and McFall, 1980). Because of the previously mentioned effects of facilitation of giant fiber conduction velocity, the interspike interval between pairs of closely spaced LGF spikes is not constant as spikes propagate along the animal (Figure 8). For spikes initiated in posterior segments, the reduction in interspike interval may be as much as 1–2 msec in anterior segments. This effect tends to further accentuate the high probability of LGF to GMN2 transmission due to longitudinal gradient effects.

4.5. Rapid Escape Movements

The rapid response of an earthworm to abrupt tactile stimulation of anterior segments consists of several components, the most obvious being a rapid and vigorous longitudinal shortening away from the stimulus. In some species the shortening is accompanied by a characteristic dorsoventral flattening and a forward pointing of setae in tail segments, which undoubtedly function to anchor the animal's tail (Bovard, 1918; Studnitz, 1937; Rushton, 1945*b*). Neural correlates and timing relationships have been examined in regard to the shortening and flattening components in some species, but there has been no study of the neural control and coordination of setal movements during escape.

In intact earthworms (*Lumbricus terrestris* and *Eisenia foetida*) tested under laboratory conditions, the firing of a single MGF spike evoked by light tactile stimulation is always accompanied by the sequential firing of GMN1 spikes and small longitudinal muscle potentials in each segment along the body, but this amount of activity is insufficient to produce rapid shortening of the body (Drewes *et al.*, 1980; O'Gara *et al.*, 1982). Likewise, GMN1-mediated muscle potentials remain small, and no shortening is observed in response to two widely spaced MGF spikes (e.g., 20 msec

interspike interval). However, when two more closely spaced MGF spikes occur (8–12 msec interspike interval), GMN1-mediated muscle potentials facilitate in anterior segments and a corresponding shortening of these segments is seen. Thus a prerequisite for MGF-mediated shortening appears to be a longitudinal muscle potential of some critical amplitude, and due to the anterior-posterior gradient in muscle potential facilitation, this shortening tends to be localized within anterior segments. Rapid shortening can occur in middle segments, but usually requires a train of three or more rather than two, closely spaced MGF spikes. Furthermore, responses in middle segments are consistently weaker and typically occur 5–10 msec later than in anterior segments. The focusing of MGF-mediated shortening into anterior segments seems reasonable because these segments are most vulnerable to attack as the animal's head is extended from its burrow.

The tail flattening that accompanies responses to anterior stimulation is also mediated by the MGF, the effectors for flattening being the septal muscles (Pallas and Drewes, 1981). As in the case with anterior shortening, tail flattening in intact animals requires at least two antecedent MGF spikes. However, for detectable flattening to occur, the spikes need not be as closely spaced. For example, some flattening may occur if the interval between a pair of MGF spikes is as long as 20 msec. A comparison of the timing of tail flattening in relation to anterior shortening has been made by Pallas and Drewes (1981). Their results from intact animals indicated that the onset of tail-flattening responses to anterior tactile stimulation occurred 1–16 msec before the onset anterior shortening, in spite of the fact that the antecedent MGF spikes were initiated in head segments. This difference in timing may enable the animal to escape more effectively because tail flattening would help anchor the animal's tail in the burrow before longitudinal shortening of anterior segments occurs. The physiological mechanisms that permit flattening responses to precede shortening are not known, but could involve differences in the electrical properties of the septal and longitudinal muscle groups or differences in the excitation–contraction coupling properties of these muscle groups.

One similarity between the shortening and tail-flattening components of escape is that the amplitude and onset latency of both components are graded in relation to the number and frequency of antecedent MGF spiking (Roberts, 1962*a*, 1966; Drewes *et al.*, 1980; Pallas and Drewes, 1981). The grading of rapid escape movements would seem advantageous for a burrowing animal that by its own locomotory movements may inadvertently encounter stimuli that are suprathreshold for giant fiber activation, but are otherwise not sufficiently threatening to require a vigorous withdrawal of the entire animal.

Based on data from studies of the MGF afferent and efferent pathways, a total reflex time can be calculated for the earliest behavioral component of escape (i.e., tail flattening). The minimal time from the delivery of a mechanical stimulus to onset of an MGF spike in the most anterior segments is approximately 5 msec. Assuming an average MGF velocity of 30 m/sec and an animal length of 150 mm, the total conduction time along the animal is 5 msec. Finally, the minimal time from the occurrence of the first MGF spike in tail segments until onset of tail flattening is approximately 15 msec (Pallas and Drewes, 1981). Thus a minimal estimate of total reflex time for rapid tail flattening in response to anterior stimulation is 25 msec, with longitudinal shortening occurring a few milliseconds thereafter.

Under laboratory conditions, the most obvious response to strong tactile stimulation of the earthworm's tail is a rapid and vigorous end-to-end shortening, which is especially pronounced in posterior segments (Studnitz, 1937; Rushton, 1945*b*; Drewes and McFall, 1980). The underlying neural basis of this shortening appears to be a high-frequency train of LGF spikes accompanied by repetitive firing of GMN2 spikes and longitudinal muscle potentials along the entire animal (Drewes and McFall, 1980). Although other movements, such as setal movements, may accompany high frequency LGF firing, these movements have not been carefully described.

The laboratory observation that vigorous withdrawal to tail stimulation is focused into posterior segments is difficult to reconcile with field observations that suggest that an anterior withdrawal occurs in response to tail stimulation. For example, Rushton (1945*b*), in describing nocturnal escape responses of worms extended from their burrows, noted that attempts to seize the worm near its posterior end (presumably within the LGF sensory field) resulted in an anterior withdrawal resembling that seen in response to touching the head. In the absence of any neurophysiological data, these observations are not easily explained, but several possibilities exist. One suggestion, by Rushton, is that posterior stimulation activates the MGF rather than LGF system due to some switchover in the escape reflex circuitry. Possibly, some type of long-lasting excitation or bias of the MGF in posterior segments could enhance or unmask MGF responsiveness to afferent inputs in these segments (Günther, 1973*b*; Smith and Mittenthal, 1980).

An alternative is that only the LGF system is activated by posterior touch, but LGF responses in the field are different than under laboratory conditions. For example, following LGF activation in the field, GMN2-mediated muscle potentials in anterior segments could be larger and more effective in triggering contraction than potentials occurring under labo-

ratory conditions. Since LGF–GMN2 coupling is known to be highly effective in anterior segments (Drewes and McFall, 1980), LGF spiking might then trigger a pronounced anterior shortening, resembling that evoked by anterior stimulation. A similar anterior focusing of LGF- and GMN2-mediated shortening may provide the basis for possible LGF-mediated escape responses to substrate vibration as the animal is extended from its burrow.

Finally, there is a possibility that posterior seizing of the animal could initiate responses mediated by both MGF and LGF systems, either simultaneously or sequentially. Sequential activation could involve some type of reafference, whereas simultaneous initiation of MGF and LGF responses could occur following substrate vibration of the whole animal. Simultaneous initiation of MGF and LGF spikes at opposite ends of the animal, in response to substrate vibration, has been often observed under laboratory conditions (C. D. Drewes, unpublished data).

4.6. Habituation

The giant-fiber-mediated reflexes in earthworms, as in other annelids, readily habituate during repeated stimulation (for review, see Dyal, 1973). Roberts (1960, 1962*b*) concluded that the main sites of MGF reflex lability were the sensory–giant and giant–motor junctions. However, Roberts' studies were done without the benefit of more recently obtained information regarding physiological properties of identifiable afferent and efferent neurons associated with the earthworm giant fiber systems (Günther, 1970, 1972; Günther and Walther, 1971). For example, coupling between MGF and GMN1 spikes remains 1:1 during trains of MGF spikes and fails only after many cycles of repetitive stimulation (Günther, 1972; Drewes *et al.*, 1980). Thus, assuming there is no intrinsic modulation of the efficacy of this coupling, the MGF–GMN1 junction does not appear to be a likely site for MGF reflex lability. On the other hand, if the MGF afferent pathway involves a preprocessing giant interneuron, as suggested by Günther and Walther (1971), then several possibilities exist for labile transmission on the afferent side of the reflex. An additional, and perhaps no less critical, site of MGF reflex lability is the GMN1–muscle junction. As shown by Günther (1972), there is an initial facilitation of GMN1-mediated muscle potentials with the first few cycles of repetitive stimulation, but this is soon followed by a pronounced and long-lasting fatigue of the muscle electrical responses. Reflex lability has not been studied in any detail in the LGF system, but could involve one or more sites in the afferent or efferent pathway, including LGF–GMN2 transmission.

Clearly, there is a need for further study of escape behavior modi-

fication in earthworms including the possibility of phenomena such as dishabituation. Future investigators in this area may look forward, either happily or with regret, to a variety of complicating factors.

1. There are two different giant fiber systems, both highly sensitive to some of the same as well as different stimulus modalities.
2. The reflexes involve at least three probable sites of lability including sensory–giant, giant–motor, and motor–muscle synapses.
3. There are important segmental variations in the functional properties of the afferent and efferent pathways of each giant fiber reflex; thus qualitative and quantitative differences in reflex lability are likely to occur in different segments of the animal.
4. Reflex properties may be modulated in relation to ongoing locomotory activity (e.g., forward or reverse peristalsis) or possibly during copulatory activity (Andrews, 1895).
5. Physiological properties of the reflex may be significantly altered by dissection procedures.
6. Intraspecific, interspecific, age-dependent, daily, or seasonal variations in reflex properties are likely to occur.

Despite this plethora of potentially complicating factors, the demonstrated capability of obtaining repeated and long-term electrophysiological assessments of giant fiber reflex function from intact animals offers some promise for systematic study of the influence of virtually all of these factors on escape reflex function and behavioral plasticity.

5. Growth and Development of Earthworm Giant Fiber Systems

Although there have been relatively few studies of giant fiber reflex development in earthworms, there appear to be several experimental advantages offered for such studies. First, anatomical studies of neuronal differentiation and maturation are favored due to the relatively large size and identifiability of the giant nerve fibers throughout development (Ogawa, 1939). Second, recordings of giant fiber spiking activity can be readily obtained from intact animals throughout postembryonic development and even during embryonic stages when giant fiber diameters are only a few microns (O'Gara *et al.*, 1982). Third, the metameric body plan offers advantages for studying longitudinal gradients in the structure and function of serially homologous neurons involved in the reflex. Fourth, earthworms possess a significant capacity for rapid regeneration of giant fibers following either specific nervous system lesions or loss of entire body segments.

5.1. Embryonic and Postembryonic Development of Escape Reflexes

The development of earthworm giant fibers has been most extensively studied in *Eisenia foetida*. Prosser (1933) noted that giant fiber pathways in *Eisenia* become histologically identifiable in anterior segments during the next to last embryonic stage, termed weak-crawling stage. During the last embryonic stage, termed normal-crawling stage, the giant fibers extend the entire length of the cord and end-to-end contractions become evident in response to tactile stimulation. These results correlate well with electrophysiological studies by O'Gara *et al.* (1982), who concluded that MGF and LGF reflex pathways are functional in normal-crawling but not weak-crawling embryonic stages. These conclusions were based on the observations that in normal-crawling embryos, MGF and LGF spikes are triggered by tactile stimulation, are conducted along the entire animal, and are accompanied by stereotyped motor activity patterns and rapid contractions (Figure 12).

Velocities and diameters of *Eisenia* giant fibers during the normal-crawling stages are MGF, 1.1–1.6 m/sec (6–7 μm) and LGF, 0.7–1.1 m/sec (2.0–3.5 μm) (O'Gara *et al.*, 1982). Although these values are considerably less than those in adult worms, numerous other features of the escape

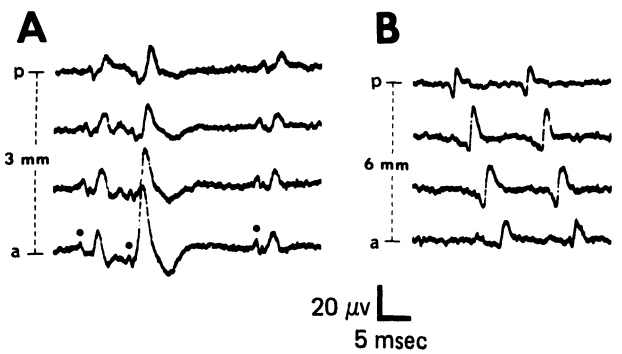


Figure 12. MGF and LGF activity recorded from the normal-crawling stage of intact embryos of *Eisenia foetida*. (A) A light tactile stimulus to the head evoked three posteriorly conducted MGF spikes (●). Each spike was followed by a larger, slower potential, presumably a GMN1 mediated longitudinal muscle potential. The interspike interval (about 7 msec) between the first two MGF spikes was sufficiently short that facilitation of the second muscle potential was seen, especially at the most anterior recording sites. (B) Light tactile stimulation to the tail evoked two anteriorly conducted, all-or-none LGF spikes. The conduction velocity of the LGF spike (about 0.9 m/sec) was slightly less than the MGF velocity (about 1.1 m/sec) (from O'Gara *et al.*, 1982).

reflex activity in embryonic worms appear adultlike. These include (1) the localization of MGF and LGF receptive fields into anterior and posterior regions, (2) a longitudinal gradient in MGF conduction velocity, (3) 1:1 coupling of MGF spikes to giant motor neuron spikes and muscle potentials, (4) an anterior-posterior gradient in facilitation of longitudinal muscle potentials during repetitive MGF spiking, and (5) occurrence of rapid shortening only in response to repeated giant fiber spiking and its accompanying large amplitude muscle potentials. The observation that each MGF spike is invariably coupled to motor neuron activity, even at about the time that MGF spikes are initially through-conducted, suggests that functional connections between MGF and its segmentally arranged giant motor neurons are completed at about the same time or before the MGF system becomes through-conducting.

The most obvious changes in the functional properties of the giant fibers during postembryonic development are age-dependent increases in MGF and LGF conduction velocity (Figure 13). Velocity increases are

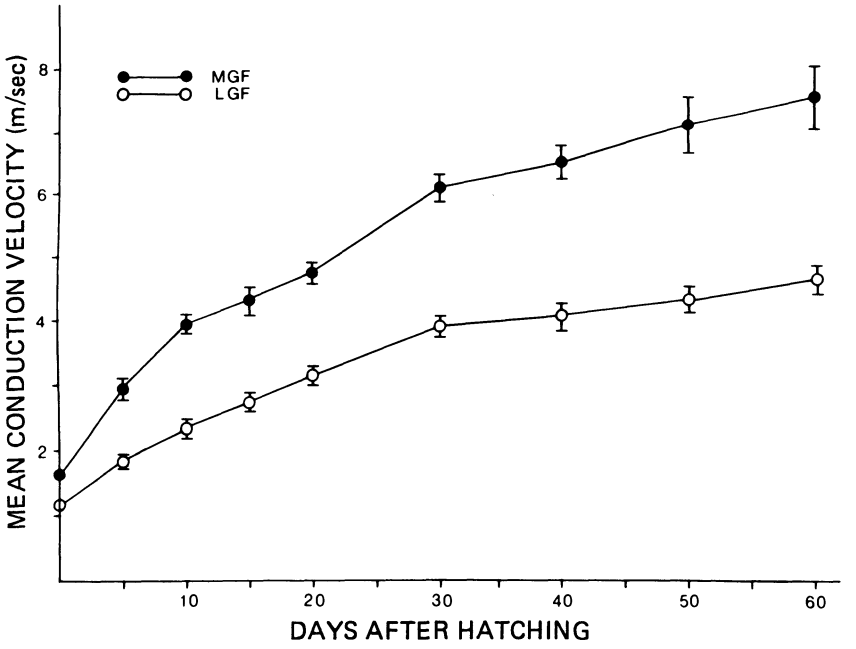


Figure 13. Relationship between giant fiber conduction velocity and age (days after hatching). Each point represents the mean velocity (± SEM) of 12 animals (*Eisenia foetida*). The same worms were tested repeatedly for the 60 days after hatching (from O'Gara *et al.*, 1982).

most rapid during the first 10 days after hatching, and then more gradual for the next 50 days or more. These velocity increases correlate with increases in fiber diameter, such that the velocity–diameter relationship appears linear for fibers of different ages. Although diameter appears to be the main factor contributing to velocity increases, it may not be the only maturational change involved. For example, age-dependent increases in sheath thickness may contribute to the observed increase in giant fiber conduction velocity. In addition, Günther (1971*a*, 1975) suggested that there may be an age-dependent dissolution of giant fiber septa during postembryonic maturation. If septal dissolution along the whole worm occurred within a relatively short time span during postembryonic development, this could account for the slight upward inflection in the age-dependent increases in MGF velocity seen between 15 and 30 days after hatching (Figure 13). On the other hand, if elimination of septa occurred gradually throughout development, then velocity increases due to septal elimination would not be easily resolved in the plots of velocity vs. age.

Aside from age-dependent changes in giant fiber function, a number of other important questions pertaining to escape reflex development remain unanswered. For example, it is not known whether there are age-dependent changes in the structure or function of the sensory neurons involved in escape reflex circuitry. Such changes might be expected in view of the scanning electron microscopic studies by Moment and Johnson (1979) that showed increases in the number and size of epithelial sensory buds during development from newly hatched to adult stages in *Eisenia foetida*. Although Moment and Johnson (1979) have suggested a possible mechanoreceptive function for these buds, there have been no studies that would implicate these structures in giant fiber escape reflex function.

5.2. Food Deprivation Effects on Giant Fiber Growth

Studies of the influence of food deprivation on growth and maturation of earthworm giant fibers are facilitated because worms are easily reared under a variety of growth conditions and because repeated assessments of giant fiber conduction parameters can be made from the same animals throughout postembryonic development. In well-fed animals, MGF and LGF velocities and diameters increase by a factor of five during postembryonic growth. By comparison, body weight increases by more than 100 times during the same period (Vining *et al.*, 1982).

As one might expect, growth of giant fibers (indicated by conduction velocity increases) as well as body weight are significantly reduced, or stunted, by food deprivation. Figure 14 shows that progressively greater

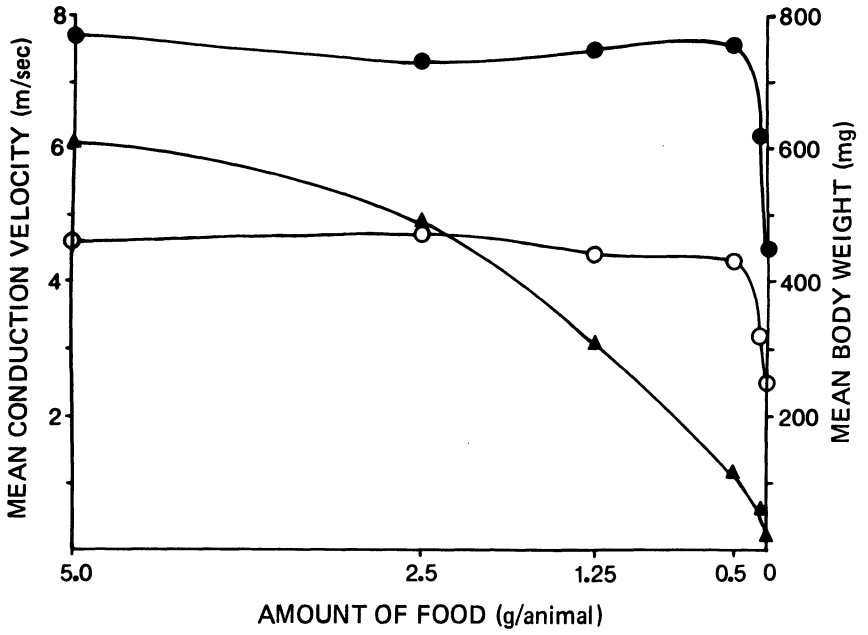


Figure 14. Effects of food deprivation on giant fiber conduction velocity and somatic growth in *Eisenia foetida*. The results were obtained from six different groups of animals (six animals/group). Beginning at hatching, each group received a different amount of food, ranging from 5 g food/worm to 0.1 g food/worm. The group means for conduction velocity and body weight were then obtained for all worms after 42 days of rearing under the various levels of food deprivation. MGF (●) and LGF (○) conduction velocities were essentially unaffected at reduced food levels ranging from 5.0 to 0.5 g food/worm, but marked decreases in velocity occurred at levels below 0.5 g. The SEM values for velocities in all groups were within the range of 0.2–0.3 m/sec for MGF and 0.1–0.2 m/sec for LGF. Body weights (▲) were markedly reduced at each food level below 5 g. Standard deviations of the body weights for the six groups, from highest to lowest food levels, were 79, 128, 96, 27, 18, and 9 mg, respectively) (from Vining *et al.*, 1982).

stunting of body weight occurred as food deprivation increased from moderate to severe levels. In contrast, stunting of giant fiber conduction velocity occurred only during the most extreme levels of food deprivation. These results suggest that there is a priority of giant fiber growth, in particular radial growth of the fibers, during periods of low food availability. The mechanisms that permit the giant fibers to be spared the growth-stunting effects of food deprivation are not known; nor is it known whether this sparing involves the entire nervous system in general or only the giant nerve fibers.

Based on experiments in which food is replenished after long periods of deprivation, it is evident that neither stunting of giant fiber conduction velocity nor stunting of body weight are irreversible (Vining *et al.*, 1982). For example, when newly hatched worms were severely food deprived for more than 60 days following hatching, marked stunting of both giant fiber conduction velocity and body weight occurred. When food was then suddenly made available, rapid somatic and giant fiber growth ensued, with growth rates resembling those seen in newly hatched, well-fed worms. In contrast, when newly hatched worms were only moderately food deprived, body weight but not giant fiber conduction velocity appeared stunted. Subsequently, replenishment of food was accompanied by rapid somatic growth, but there was no concomitant increase in giant fiber conduction velocity. This provides further evidence for the independent control of somatic growth and giant fiber growth.

Despite the stunting of giant fiber conduction velocity during extreme food deprivation, the capability of giant-fiber-mediated reflex responses to tactile stimulation persisted for many weeks in starving worms (Vining *et al.*, 1982). This maintenance of escape reflex function during food deprivation might be expected in view of the wide variation of food availability that is likely to occur in nature and the importance of the rapid escape reflex to the worm's survival.

5.3. Regeneration of Giant Fibers

One factor contributing to the rapidity of giant nerve fiber regeneration in annelids is the lack of significant giant fiber degeneration in segments anterior and posterior to a ventral nerve cord lesion, a situation undoubtedly related to the segmental origin of the interneurons forming the giant fiber systems. This lack of degeneration has been demonstrated in the giant fibers of several species of earthworms (Bovard, 1918; Hall, 1921; Birse and Bittner, 1976, 1981), in polychaete giant fibers (Nicol, 1948c; Hulsebosch and Bittner, 1981), and in the S cell of leeches (Frank *et al.*, 1975; Carbonetto and Muller, 1977). In the case of leeches, the failure to degenerate includes not only the S cells in segments adjacent to the lesion, but also the distal stump of the specific S-cell axon that is severed (Frank *et al.*, 1975; Carbonetto and Muller, 1977).

The survival of giant fibers in segments anterior and posterior to a lesion creates a favorable situation for successful regeneration because the distances over which reconnecting fibers must grow are relatively short, often less than 1 mm. It should be noted, however, that failure of giant fiber degeneration in segments adjacent to a lesion does not nec-

essarily ensure successful regeneration, as shown in several species of polychaetes (Nicol, 1948*c*; Hulsebosch and Bittner, 1981).

When regeneration does occur, it generally involves outgrowth of multiple processes from one end or in some cases from both ends, of the severed fiber (Frank *et al.*, 1975; Carbonetto and Muller, 1977; Birse and Bittner, 1976, 1981; Hulsebosch and Bittner, 1981). It is these small-caliber processes that reunite the severed ends of the fibers and form the anatomical substrate for re-establishment of spike conduction across the lesion. As recovery progresses, these processes may increase in diameter and decrease in number (Birse and Bittner, 1981).

In earthworms, the time required for establishment of successful spike transmission across the lesion is variable and depends on individual variations in the regeneration process and on testing procedures employed. Use of noninvasive electrophysiological recording methods has shown that recovery of spike through-conduction occurs very rapidly, especially in the MGF. In *Lumbricus terrestris*, MGF through-conduction is re-established as early as 5 days and usually within 7 days after severing the giant fibers (Balter *et al.*, 1980; Pallas and Drewes, 1981). Even more rapid recovery is seen in *Eisenia foetida*, in which through-conduction of MGF spikes is re-established as early as 20 hr and usually within 48 hr after severing the ventral nerve cord (Vining and Drewes, 1982). During the initial period of functional recovery, properties of spike transmission across the lesion in *Lumbricus terrestris* include: (1) intermittently successful spike transmission across the lesion, (2) conduction delays of several milliseconds within the lesioned area, (3) occurrence of "rebound" spikes (Figure 15), and (4) similarity of conduction properties in both directions across the lesion (Balter *et al.*, 1980). Interestingly, all of these properties are highly reminiscent of the transmission properties associated with the small-diameter transverse junctions that normally connect pairs of nonregenerating giant fibers in polychaetes (Bullock and Turner, 1950; Bullock, 1953; Mellon *et al.*, 1980). After several weeks of recovery, spike transmission across lesioned earthworm giant fibers appears more normal, as indicated by an increased tendency for 1:1 spike transmission across the lesion, a reduction in the conduction delay within the lesion, and a decreased tendency for rebound spiking (Balter *et al.*, 1980).

The *in vivo* conduction properties of regenerating earthworm giant fibers, as determined by Balter *et al.* (1980), do not coincide exactly with *in vitro* properties of regenerated giant fibers, as determined in isolated ventral nerve cord preparations (Birse and Bittner, 1976, 1981). In the latter studies, somewhat longer times were required for successful through-conduction, and spike transmission was often not bidirectional. These differences between *in vivo* and *in vitro* performance of regenerated giant

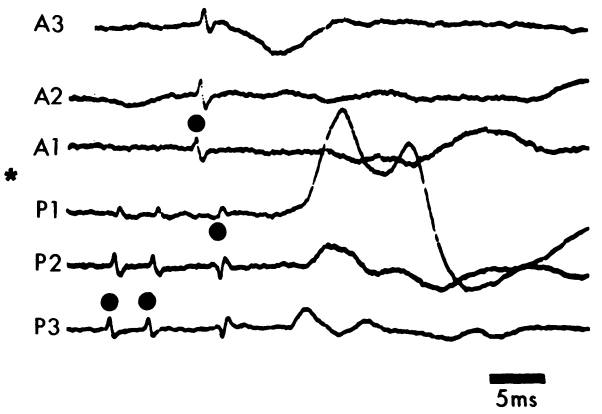


Figure 15. Spiking activity in the regenerating LGF of *Lumbricus terrestris* 14 days after ventral nerve cord transection. The LGF spiking was initiated by tactile stimulation of the tail and recorded at six sites along the ventral surface of the intact animal. The three recording sites A1–A3 were just anterior to the regeneration site (*), whereas the other three P1–P3, were just posterior to the regeneration site. The distance between adjacent recording sites was 4 mm. The response to touch consisted of an initial pair of LGF spikes (dots in P3) that were conducted toward the regeneration site. The first spike failed to conduct across the site of regeneration, whereas the second spike was successfully conducted across after a delay of approximately 3 msec (dot in A1). An LGF rebound spike (dot in P1) was initiated approximately 2 msec after the spike at A1. The waveform of the rebound spike was predictably inverted since it was conducted posteriorly. Intermittent spike conduction, conduction delays, and rebound spiking were also characteristic of the regenerating MGF (from Balter *et al.*, 1980).

fibers are likely an indication that the trauma associated with nerve cord isolation reduces the efficacy of spike transmission across the newly formed and relatively labile connections joining the severed giant fibers. This idea is supported by noninvasive testing of giant fiber through-conduction in *Lumbricus terrestris* at various times following ventral nerve cord transection (E. P. Vining, unpublished data). Such recordings have shown that even after through-conduction of giant fiber spikes is re-established across the regenerated portion of the nerve cord, a blocking of through-conduction at the transection site can be readily induced by slight trauma elsewhere in the animal, such as a dorsal puncture or cut in the worm's body wall.

Despite these effects, there seems to be uniform agreement from *in vivo* and *in vitro* studies that there is a high degree of precision and specificity with regard to the re-establishment of connections between the severed fibers; that is, anterior and posterior portions of a severed MGF always reconnect to one another, as is the case for the LGF. Based on

electrophysiological recordings from regenerating giant fibers, there is also general agreement that transmission between regenerated earthworm giant fibers probably involves some type of electrical junction (Balter *et al.*, 1980; Birse and Bittner, 1976, 1981). Biophysical and ultrastructural studies of giant fibers during early stages of regeneration would help to confirm and further resolve this issue.

In addition to the earthworm's capabilities for regeneration after a simple transection of the ventral nerve cord, there is evidence that giant fiber regeneration can occur after ablation of one to three segments of ventral nerve cord (Bovard, 1918; Birse and Bittner, 1981). Except for the longer time required for recovery, the regeneration process appears similar to that seen after transection. That is, highly specific reconnection of giant fibers occurs via outgrowth of multiple processes arising from the severed ends of the fibers. Birse and Bittner (1981) found no evidence for reformation of the ablated cell bodies in earthworm giant fibers. This also appears to be the situation in the polychaete, *Nereis*, although reformation of giant fiber cell bodies apparently does occur in another polychaete, *Clymenella torquata* (Hulsebosch and Bittner, 1981).

The compensatory regeneration of whole body segments at the site of removal of head or tail segments has been well documented in many classical studies involving earthworms and polychaetes (for older reviews see, Stephenson, 1930 and Hyman, 1940). From the standpoint of developmental neurobiology, the capability for new segment formation raises interesting questions in regard to the interfacing and interaction between the newly formed and original portions of the nervous system, as well as influences of axial gradients on nervous system and segment differentiation. Recently, some of these questions have been addressed in a preliminary study of the electrophysiological properties of giant fibers in regenerating tail segments of *Eisenia foetida* (Drewes and Marty, 1982). By nine days after removal of 36–44 tail segments, a delineation of new segments has occurred in the tail bud and by 14 days all-or-none LGF spikes can be noninvasively detected in bud segments in response to tactile stimulation of either bud segments or older adjacent segments. The LGF conduction velocity in bud segments at this time is nearly identical to that of embryonic worms at the normal crawling stage. Subsequent bud growth involves increase in bud segment size but no increase in segment number (Moment, 1979). During this growth the rates of increase in LGF velocity are nearly identical to those seen during normal development, suggesting that the processes underlying giant fiber growth in regenerating tail segments recapitulate those occurring during normal embryonic and postembryonic development.

Interestingly, tail regeneration is not necessarily restricted to con-

ditions of posterior segment removal, but can occasionally occur following removal of anterior segments (Morgan, 1899; Gates, 1950). In such cases these misplaced, or so-called "heteromorphic" tails appear to be exclusively subserved by the LGF sensory field (Drewes and Marty, 1982). This indicates that formation of LGF sensory field in regenerated segments can occur independently of the location of regeneration along the body axis.

6. Conclusions

Rapid escape reflexes in polychaetes, leeches, and oligochaetes are mediated by one or several relatively large-caliber and through-conducting giant fiber networks. The ensemble of neurons involved in generating and processing afferent inputs to the giant fibers, as well as the motor neurons controlling escape movements, consist of serially repeated, identifiable neurons. Appropriate variations in stimulus sensitivity and escape reflex movements along the length of the animal are achieved by segmental gradients or transitions in the properties of both peripheral and central components in the reflex. Although some progress has been made in characterizing the reflex circuitry and segmental variations in reflex function in a few annelids (e.g., leeches and earthworms), we have a very limited understanding of escape reflex organization in the vast majority of annelid species.

Several features pertaining to escape reflex development and regeneration have been revealed in studies of oligochaete earthworms. For example, noninvasive electrophysiological recordings indicate that many adultlike features of earthworm escape reflex function are established in late embryonic stages and are maintained throughout postembryonic maturation. In under-nourished worms postembryonic growth of giant fibers, and possibly growth of other neuronal elements, are given priority in relation to somatic growth. Finally, very rapid regeneration of earthworm giant fibers and escape reflex function occurs following either specific nervous system lesions or loss of entire body segments.

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